Genomic variation of Salmonella serovar Infantis

Litrup, Eva; Kiil, Kristoffer; Zhou, Zhemin; Sørensen, Gitte; Guttman, David; Torpdahl, Mia; Achtman, Mark; Møller Nielsen, Eva

Published in:
Med-Vet-Net Association International Scientific Conference DTU, Lyngby, Denmark, 24-25 June 2013

Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

MED·VET·NET ASSOCIATION

Med-Vet-Net Association International Scientific Conference
DTU, Lyngby, Denmark, 24-25 June 2013

ABSTRACT BOOK

www.medvetnet2013.eu

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Preface

We warmly welcome all delegates to the 2nd Med-Vet-Net Association Scientific Conference. Zoonoses and the One Health – One Medicine theme have gain a high profile over the past decade and research in these areas continues to grow. The "One Heath - One Medicine" theme chosen for our conference was embraced by our predecessor, the Med-Vet-Net FP6 network some years ahead of its time and is a concept seemly developed for the association!

The Med-Vet-Net Association was officially launched in October 2009 with the aim of becoming the EU FP6 network of Excellence Med-Vet-Net's most enduring legacy. The Association has linked its very purpose to continuing Med-Vet-Net's success through its overarching aim to increase, capitalize and disseminate scientific knowledge on zoonoses with the main emphasis on food-borne zoonoses. The self-funded Association, currently comprises 15 scientific partners and aims to build on the success of its predecessor by strengthening existing partnerships and forging new collaborations both within Europe and around the world.

Central to the Med-Vet-Net Associations goals is the dissemination of scientific knowledge and experiences both between scientists within the network as well as externally to the public, media and policy makers. Much of this information is provided electronically via a public website (www.mvnassociation.org). The association also has roles in training and facilitating the coordination of cross disciplinary EU bids between partner institutes.

Since the last scientific meeting the Association has welcomed three new members the Polish National Veterinary Research Institute (PIWET), Veterinary and Agrochemical Research Centre (VAR) and the Norwegian Veterinary Institute (NVI). The Association has also funded seventeen short term scientific missions and two workshops. For this the 2013 ASM meeting it has been particularly rewarding to see abstracts from recipients of these scientific missions from our member institutes and also from international delegates, some of whom are also part of National or International Zoonoses networks.

The conference has attracted significant interest with 80 abstracts being accepted, all of an extremely high standard. We are indebted to the Conference Scientific committee for reviewing the abstracts and without their support we would have been unable to deliver such an interesting scientific programme.

For the 2013 ASM we are extremely fortunate to have three keynote and a further three presentations and by distinguished scientists, who will highlight medical and veterinary perspectives of the one health concept and provide examples of scientists working in medical, veterinary and food-based institutions can work together sharing different challenges for combating zoonoses. We have a departure from the Med-Vet-Net NoE format with the introduction of invited expert speaker slots from the Med-Vet-Net Association Institutes to open and close the four main sessions. Furthermore, we have also introduced a poster pitching sessions. These will highlight work from submitted abstracts that we hope will be of particular interest and will emphasise the work of young scientists. Subject areas for the main conference sessions, all of which have a one-heath angle, include exploitation of next generation sequencing to determine molecular epidemiology, risk modeling, epidemiology of antimicrobial resistance, new tools for detection and control, epidemiology and ecology of pathogens, host pathogen interactions and host responses. These are highly topical and reflect current concerns to the health of humans and welfare of animals. We hope that the oral presentations and posters alike will be of interest not only to the specialists in these particular areas, but to all delegates present in Lyngby.

Considerable planning has gone into making the meeting a success, both scientifically and socially. I would like to thank our Danish Technical University hosts, Biopeople, in particular the support of David Featherston, the Scientific Committee and the Association's Governing Board who have contributed immensely to the organisation of this conference. I would also like to acknowledge the financial support from our sponsors which has been used to support this event.

I hope that you will participate in our social networking events. We have arranged a beer and sushi networking event following the close of the scientific programme on day one. The Gala Dinner will take place at the Royal Danish Theatre, where world-renowned new Nordic cuisine will be combined with delicious wines, interesting dialogues and beautiful water-side views.

On behalf of the Organising Committee I look forward to meeting you in Lyngby for a highly enjoyable and productive conference.

Roberto La Ragione
President of the Med-Vet-Net Association

KN01*: Global outreach from EU to developing countries

Marta Hugas[1], Franck Berthe, Tobin Robinson and DjienLiem

European Food Safety Authority (EFSA). Via Carlo Magno, 1 A. Parma 43126 Italy
[1]Corresponding author

Food safety has no borders, and with increasing globalisation this has become more and more evident. There are examples showing the role of the EU in the past in exporting food safety issues from the EU to the rest of the world (e.g. BSE), but there have also been other examples of risks coming from the wider world into the EU (e.g. vector-borne diseases). It becomes evident that the efforts to maintain EU's high standards of food safety and human health protection may become unsuccessful if a global view is not kept as a priority. In this sense networking is crucial as it is the need to work cross-disciplinary and towards a One Health approach allowing the bridging of the gap between the human and veterinary sides including the consideration of multiple angles and facets and avoiding thinking in boxes across the food chain.

EFSA is committed through its founding regulation to contribute to the prevention of emerging risks in the field of food and feed safety. The early identification of drivers leading to future scenarios for the identification of emerging issues is a difficult but crucial task. In the case of zoonoses this involves the BIOHAZ and AHAW Panels under the coordination of the Scientific Committee. The approach proposed for biological hazards includes expert consultations with the above panels, followed by an expert workshop. The success can only come by networking with all partners from EU to the global community.

***Marta Hugas** joined EFSA in 2003 as a scientific coordinator of the BIOHAZ Panel. In 2006 she was nominated Head of the Biological Hazards Unit leading a team of staff supporting the BIOHAZ Panel. Previously she was Head of the Food Microbiology and Biotechnology Unit at IRTA (Institut de Recerca I TecnologiaAgroalimentaries from Catalonia in Spain) where she was leading a research team on meat safety.*

KN02*: Animal and public health surveillance of infectious diseases

Frank M. Aarestrup

National Food Institute, Technical University of Denmark
Kemitorvet
Building 204
2800 Lyngby
Denmark

Infectious disease is a major burden for both human and animal health. The exact impact is difficult to quantify, but for humans alone infectious diseases directly account for >25% of all global deaths. Amongst others because of increased travel, trade and populations densities, animal and human populations worldwide are increasingly confronted with the threat of existing, new and emerging infectious epidemics spreading faster and appearing more frequently than ever before. Modern demographic, environmental, technological and societal conditions favour the spread of these epidemics at a global scale.

Besides their direct impact, infectious epidemics in both animals and humans can have major economic consequences. Thus, novel findings of diseases in animal populations can have drastic consequences for global trade as observed with the recent influenza cases in livestock in Denmark and The Netherlands. As for the food sector, foodborne outbreaks can unsettle consumers' trust and have negative effects on trade and the economy of the sector. Furthermore, human disease outbreaks can change travel preferences and frequencies as observed with SARS and recent coronavirus cases.

This raises new challenges beyond national borders to public health, veterinary and food safety scientists and experts, policymakers, and populations. Especially, the need for global real-time monitoring of, in principle, all infectious agents is essential. This includes the ability to detect new, emerging and re-emerging epidemics, determine the global burden of disease, detect effects of interventions and to guide both research and areas for control.
Today, monitoring in many countries is performed separately in veterinary and human laboratories, meaning that data is not collected in a harmonised and standardised way and is often only made available after some time delay.

The rapid developments in next-generation sequencing give high hopes that we, in the near future, might have a technology that can change this situation. Whole genome

and whole community sequencing can rapidly generate almost complete data that has the potential to be exchanged and compared globally in a standardised way. Several studies have already shown the great potential of the technology. There is, however, a major need for the development of standards for sample collection, preparation and analysis, as well as for data collection, management and sharing. Furthermore, procedures and infrastructures, both physical and intellectual, for storage of information and interpretation need to be developed and defined.

The potential of the technology, some examples on the usage and potential structures for a global system, or set of systems, will be presented and discussed.

KN03*: The societal costs of the Dutch Q fever outbreak: evaluation of past and future control strategies

G. Morroy[1] and M.A.P.M. van Asseldonk[2]

1 *Municipal Health Service, Infectious diseases, Den Bosch, The Netherlands*
2 *Economic Agricultural Research Institute (LEI) of Wageningen UR, Wageningen, The Netherlands*

Background
In the Netherlands, more than 4,000 human Q fever cases, including 25 fatalities, were notified during the 2007–2010 Q fever outbreak. Veterinary control measures were introduced reluctantly, late and gradually, fearing economic damage to the sector. However, within three years 60,000 dairy goats were culled.

Past lessons
Accounting for 85%, human costs are spread over a decade whilst veterinary costs are proportionally small and immediate. Humans develop late complications such as Q fever fatigue syndrome or chronic Q fever, which negatively affects quality of life and productivity.

Future
By analysing disease dynamics, we developed a Q fever transmission model. Compared to culling or breeding bans for Q fever infected dairy goat farms, costs of retaining the preventative vaccination programme are relatively low. This vaccination programme on Q fever free farms is preferred if the probability of re-infection exceeds once every 15 to 20 years. Only the absence of Coxiella burnetii from both livestock and the environment would warrant a return to non-vaccinated herds. Unfortunately, much remains unknown on the probability and mechanisms of re-infection of goat herds.

Conclusion
Q fever poses a serious long-term burden on patients and society. The real impact of a zoonosis outbreak only becomes apparent when combining human health, societal and veterinary costs. Veterinary costs are immediate, apparent and proportionally small. Due to a trickle-down effect over a decade, human cost and societal implications are underestimated. Finding the balance between economic livestock interests and human health remains a challenge when dealing with outbreaks of zoonotic diseases.

Co-authors:
D.M. Bontje, J.A. Backer, H.I.J. Roest, H.J.W. van Roermund. Central Veterinary Institute (CVI) of Wageningen UR, Lelystad, The Netherlands.
R.H.M. Bergevoet, Economic Agricultural Research Institute (LEI) of Wageningen UR, Wageningen, The Netherlands.
J. Prins, SEO Economisch Onderzoek, Arbeid & Onderwijs, Amsterdam, The Netherlands.
W. van der Hoek, National Institute for Public Health and the Environment, Centre for Infectious Disease Control, Bilthoven, The Netherlands.
J. J. Polder; National Institute of Public Health and the Environment, Unit Population, and Tilburg University , Tilburg, The Netherlands.

Dr Gabriella Morroy *is a medical consultant in communicable disease control at a large municipal health authority ('s-Hertogenbosch) in The Netherlands. As part of a PhD project, she researches the costs of the Dutch Q fever outbreak. Studies include the long-term health status, productivity loss, health-care consumption and serological follow-up of Q fever patients.*

Dr. Marcel van Asseldonk *is a senior researcher in risk management at the Agricultural Economic Research Institute, part of Wageningen University. He supervises and conducts research in the field of risk analysis and risk financing in agriculture. His research area focuses on economic assessment of animal diseases, public-private livestock funds and livestock insurance schemes.*

IS01*: OIE perspective on One Health: Sharing challenges for combating zoonoses

Elisabeth Erlacher-Vindel[1], **Susan Corning** [2]

1 *Deputy Head,*
2 *Interim Project Coordinator*
Scientific and Technical Department, World Organisation for Animal Health, (OIE),
12 Rue de Prony, 75017 Paris,
France

Established in 1924, the World Organisation for Animal Health (OIE) has a mandate from its 178 member countries to improve animal health and welfare worldwide, making an important contribution to the prevention and control of emerging and re-emerging animal diseases, including zoonoses. Through the support of its worldwide network of 284 reference laboratories and collaborating centres and its specialist commissions, the OIE provides scientific knowledge and guidance to OIE member countries as well as to global stakeholders.

Engaging abroad global stakeholder collaboration has become critical to pandemic preparedness. With an accelerating movement of commodities and people throughout the world, pathogens are being transported at an unprecedented rate and scope. As approximately two-thirds of human pathogens are zoonotic, it is critical to have in place a global strategy to collaboratively prevent and manage health risks at the human-animal-environmental interface. The recent avian influenza (HPAI H5N1) pandemic motivated the urgency of a plan to share the challenges of combating zoonotic diseases through a cross-sectoral approach. In support of the 2008 interagency strategic framework, *'Contributing to One World, One Health – A strategic framework for reducing risks of infectious diseases at the animal–human–ecosystems interface*', the OIE, FAO and WHO formed a tripartite partnership to jointly address such important global health risks. This multidisciplinary One Health approach has broadened and facilitated regional and national capacities for early detection, improved laboratory-based disease diagnosis, rapid disease response and risk reduction of zoonotic and other diseases. Together, the tripartite has shared information, established joint responsibilities, and developed a high-level technical perspective to address serious health challenges such as those posed by avian influenza, rabies, and antimicrobial resistance. By working together with its counterparts, the OIE strives to improve the capacity of individual countries to detect and manage zoonotic diseases, and thus mitigate their evolution into serious global health risks.

Dr Susan Corning *has had a career -long commitment to furthering global collaboration within and between the animal and human health sectors. Originally from the USA, she graduated from Onderstepoort School of Veterinary Medicine in South Africa. Dr Corning started her career as the Southern Africa Regional Development Director for Shell International Animal and Public Health, and later held senior roles in the pharmaceuticals and animal welfare sectors in the UK and Europe.*
A Fellow of the Royal Society of Public Health, she has also held CEO positions within international medical relief and global health philanthropy organisations. As a One Health consultant, Dr Corning has led international projects in stakeholder engagement and disease management. She is currently working within the Technical and Scientific Department of the World Organisation for Animal Health (OIE) as a project coordinator, guiding the development and facilitation of cross-sectoral approaches to address health risks at the human-animal interface.

IS02*: Detection and control of infections shared with wildlife

Christian Gortazar

Head of the Health and Biotechnology research group SaBio, National Wildlife Research Institute IREC (CSIC-Univ. Castilla – La Mancha), Ronda de Toledo s.n. 13071 Ciudad Real, Spain

The control of infections shared with wildlife requires the development of strategies that reduce pathogen transmission between wildlife and domestic animals and between wildlife and humans. Setting up a proper disease and population monitoring scheme is the absolute priority before even making the decision of whether or not to intervene. Thereafter, disease control can be achieved by different means, including (1) preventive actions, (2) arthropod vector control, (3) host population control through random or selective culling or through habitat management and (4) vaccination. The alternative options of zoning or no-action should be considered too, particularly in view of a cost/benefit assessment. Ideally, tools from several fields should be combined in an integrated control strategy. The success of disease control in wildlife depends on many factors, including the nature of the disease and the characteristics of the pathogen, the availability of suitable diagnostic tools, the characteristics of the wildlife host(s) and vectors, the geographical spread of the problem, the scale of the control effort and the attitudes of the stakeholders. This presentation will introduce examples on wildlife disease and wildlife population monitoring in Europe based on the APHAEA network (http://aphaea.eu/).

IS03*: Control of animal trichinellosis, development of new serological tests and vaccines

Isabelle Vallée, SA Lacour, P Boireau

JRU BIPAR, Anses Maisons-Alfort, Laboratory for Animal Health, France

Trichinellosis is a foodborne zoonotic disease caused by infection with the nematode *Trichinella* spp. All monogastric animals are sensitive to this parasite that can be transmitted by consumption of raw or undercooked contaminated meat. Detection of *Trichinella* larvae in sensitive animals entering the food chain (mainly pigs, horses and wild boars) is under EU regulation. The only in use reference method is a direct test based on an artificial digestion of muscle samples performed routinely at the slaughterhouses and requiring a high level of technical performance. However, serological tests may be used when validated, for epidemiological surveillance of pig livestock, which will avoid the current costs associated with the direct test which requires use of pigs raised under controlled farming conditions, which are, at the current time, considered to be at negligible risk of contamination.

Our research group is working on improvements of the direct test and mainly in the implementation of ring tests for evaluation of a laboratory's performance by an original method of a large-scale production of proficiency samples. In parallel, we also aim to develop new serological tests based on identification of antigens expressed during early parasitic stages of *Trichinella spiralis*. Indeed, we evidenced the usefulness of recombinant proteins expressed during the intestinal phase of the parasite life cycle for ELISA tools. The prototype ELISA exhibits a reduced blind-window in detection of seropositive pigs compared to the only available test based on excretion/secretion products. Finally, a recombinant vaccine inducing a high level of protection against *T. spiralis* infection in pigs was developed and could find an application in breeding exposed to *Trichinella*.

IS04*: Host-pathogen interactions in inflammatory bowel disease

Andreas Munk Petersen

Hvidovre University Hospital, Department of Gastroenterology, Kettegård Alle 30, 2650 Hvidovre, Denmark

During the past decades lifestyle-associated diseases such as atopic dermatitis, asthma, ulcerative colitis, Crohn's disease and arthritis have increased in incidence in the Western world. Nevertheless, the mechanisms behind those events are still unclear. Overall inflammatory bowel disease (IBD) would seem to be the result of three major influences; host genetic risk factors, environmental factors and immunological factors. The exact interplay between these factors is only partly elucidated in IBD. It is postulated that the increase in IBD cases is the result of "westernisation" of lifestyle, such as changes in diet, smoking, pollution and industrial chemicals. All these factors could have their effect through an underlying change in the gut flora. The role of gut microbiota in the development of IBD is generally accepted. However, specific intestinal triggers for IBD are so far unknown. Current knowledge on genetics and microbiota changes in IBD will be presented with a special focus on the role of specific IBD-associated *E. coli*.

IS05*: Zoonotic virus and species barrier crossing: example of hepatitis E virus

Nicole Pavio

Virology Unit, Animal Health Laboratory Anses, Maisons-Alfort, France

Hepatitis E virus (HEV) is responsible for enterically transmitted acute hepatitis in humans. In 1 to 4% of the cases it leads to fulminate hepatitis, and this percentage can reach up to 20% in pregnant women from endemic regions. Among all hepatitis viruses, HEV is unique as it has several animal reservoirs. HEV genotypes 1 and 2 infect exclusively humans while genotypes 3 and 4 were detected both in human and other animals. HEV can be found in wild board, deer and rabbit, but the major animal reservoir of HEV worldwide is domestic pig. Several lines of evidence indicated that animal to human transmissions occur. First, animal models were developed in non-human primates and swine confirming that cross-species infections can occur with HEV genotypes 3 and 4. Second, individuals with direct contacts with animal reservoirs are at high risk of HEV infection. Furthermore, consumption of infected meat has been associated with clinical cases, thus, hepatitis E is also a foodborne disease. Pork liver and other products containing pork liver have been shown to contain HEV RNA. HEV has a high genetic variability and it is not known whether all HEV strains are zoonotic, with transmissions dependant from ecological factors, or if only some variants are capable of crossing the species barrier. Studies are ongoing to elucidate mechanism of HEV host restriction.

IS06*: Assessing state and federal responses in USA to simulated introductions of Rift Valley fever virus

Professor Paul Gibbs

College of Veterinary Medicine
University of Florida
Gainesville, Florida, USA

The introduction of West Nile (WN) virus into New York in 1999 and the number of cases of encephalitis in people, horses and indigenous birds came as a surprise to the USA. The similarity of the epidemiology of WN virus and Rift Valley fever (RVF) viruses indicates that, were RVF virus to be introduced to the USA, the ensuing epidemic could have a greater impact on human and livestock health than WN. Experimental infections of North American species of mosquito with RVF virus have indicated that several common and widely distributed species could transmit the virus. To test the ability of state and federal agencies to respond to an introduction of RVF virus into the USA, three separate "table top" exercises have been held in Florida, Puerto Rico, and St Croix. The timeline of the exercises was real-time for the first 1½ days of the scenario and accelerated on the second half of day 2 into the following 3 weeks. The exercises provided the participants with the opportunity to plan, initiate, and evaluate current response concepts and capabilities within the context of One Health. These exercises can be considered an example for future multi-agency exercises dealing with a vector-borne disease with a) a zoonotic component and b) involving ruminant wildlife. The exercises highlighted a) that RVF might be very difficult to control and b) the need for more information on the susceptibility of North American wildlife species to Rift Valley fever virus.

IS07*: Integrated epidemiological and microbiological research on livestock and health; a One Health approach

Yvonne van Duynhoven

Head of Centre for Zoonoses & Environmental Microbiology
National Institute for Public Health (RIVM) - Centre for Infectious Disease Control,
Bilthoven, The Netherlands

In The Netherlands, the unprecedented large Q fever outbreak in 2007-2009 and the more persistent problem of the emergence of livestock-associated antimicrobial resistant microorganisms fueled the sense of urgency for the One Health approach. New initiatives included the development of an integrated human-veterinary zoonosis structure (covering among others a human-veterinary early warning, risk evaluation and outbreak response), increased regional and national consultation and information exchange between veterinary and human health professionals, One Health education for academic students and a general aim for a more human-veterinary integrated approach in preparedness, surveillance, policy-making and research. Because of the massive health impact of the Q fever outbreak and the drastic control measures, extensive political and public debate specifically addressed the future developments in livestock industry in our country. This debate made clear that in future developments, next to the main historical driving forces of livestock industry in food supply, food security and economic values (trade, profit), public health, animal health and welfare and land-use planning should be taken into consideration to achieve a sustainable livestock industry. For that, expanding interdisciplinary collaborations in all aspects of care for humans, animals and the environment is a prerequisite as well as improved awareness of the highly complex multi-dimensional character of livestock farming with sometimes conflicting stakes. Currently, well-informed, science-based choices from a health perspective are largely hampered by the lack of information on the possible relationships of (different types of) livestock farming with human and animal health and welfare. Especially, the possible health risks for residents living in the proximity of livestock farms needs to be elucidated, as research in this field is very scarce. Traditionally, studies on health risks

from animal production were focused on workers in the food chain. The lecture will outline the above-mentioned context, summarise the scarce data on health risks for residents around livestock farms, the planned research in that field as well as give examples of integrated epidemiological and microbiological studies and surveillance activities targeting both humans and animals or their living environment.

IS08*: High through-put molecular characterisation of the bacterial resistome

Muna Anjum

Department of Bacteriology and Food Safety, Animal Health and Veterinary Laboratories Agency, New Haw, Surrey, United Kingdom

The use of high through-put molecular characterisation approaches for molecular epidemiology has become increasingly common in the past decade. The molecular methods used range from the use of PCRs, microarrays to whole genome sequencing. In an EU FP7 project (Antiresdev) we used an expanded microarray that is able to detect more than 90 different antimicrobial resistant genes present in Gram-negative bacteria to detect resistances present in >3500 Gram-negative bacterial isolates collected from faeces and saliva, to look for the effect of antibiotic treatment on the resistome. Bacterial isolates were cultured from samples collected up to 1 year after treatment of healthy volunteers treated with different antibiotics and compared against samples collected from the placebo group over the same time period. Our results suggest that Gram-negative bacteria such as *Escherichia coli* are prevalent in the human gut and many are naturally resistant to several antibiotics. There was some effect of antibiotic administration, although it was not sustained. These resistance genes are possibly present on mobile elements such as plasmids that are able to move around. Curing of plasmids was shown to affect the ability of strains to grow in the presence of antibiotics and stress modulators indicating that they may be associated with fitness and adaptation to different environments. Furthermore, these *E. coli* isolates of human origin were able to colonise chickens and there was a possible transfer of antibiotic resistance *in vivo* from the challenged strains to other Gram-negative bacteria.

IS09*: Data for microbiological risk assessment: past, present and future

Emma L. Snary*& Andrew Hill

Animal Health and Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey, United Kingdom

*Emma.snary@ahvla.gsi.gov.uk

"*Rubbish in; rubbish out*" is a phrase often used when describing data needs for the development of mathematical models, including microbiological risk assessments (MRAs). However, since the evolution of MRA in the 1990s the demand for data for the parameterisation of these models has increased due to the demand for, and complexity of, MRAs in more recent times. Such models require data from many fields, including epidemiology, microbiology, medicine, agriculture and livestock/food production. However, how much of the data currently being produced and published within the scientific literature is directly or indirectly useful for MRA? Good communication between risk assessors and data generators is essential if there is not to be a discord between the data risk analysts needs and what data generators *think* we need. Within this presentation, using examples of MRA work carried out within the AHVLA, I will reflect on many of the data issues experienced within this area. The relative merits of the data from the past and present for use in MRA will also be touched upon and, finally, some thoughts on what the future might hold.

IS10*: The development of probabilistic graphical models to assist on strategic decisions for the control of Campylobacter in poultry

Ana B. Garcia

Division of Epidemiology and Microbial Genomics, National Food Institute, Technical University of Denmark, Mørkhøj Bygade 19, 2860 Søborg, Denmark

Human infections with Campylobacter constitute an important public health problem, and poultry has been identified as a significant risk factor. Risk assessment models indicate that a reduction of human campylobacteriosis cases could be achieved by the successful implementation of efficient Campylobacter control strategies in poultry. Poultry producers need to make difficult decisions under conditions of uncertainty regarding the implementation of Campylobacter control strategies. Decision support systems might assist producers in selecting cost-efficient control measure(s) they should implement to control Campylobacter. Probabilistic graphical models (PGMs) use Bayesian methods to include probability distributions in models that integrate knowledge in just one representation. PGMs may include hundreds or even thousands of variables and represent complicated relationships among them using conditional probability distributions.

At the Med-Vet-Net conference, we present PGMs designed in order to assist the poultry industry to make strategic decisions for the control of Campylobacter under conditions of uncertainty. The type of PGM that we use is an influence diagram that has two components: a qualitative and a quantitative part. The qualitative part is represented by a directed acyclic graph (DAG) formed by diverse "nodes" such as variables, decision nodes and utility functions as well as the relationships between them. The quantitative part of the models includes mathematical expressions and probability distributions associated with the nodes. The solution of an influence diagram is a strategy (selected control(s)- decreased risk- financial balance). The strategy is determined using the principle of maximising expected utility based on selecting a decision that will offer the greatest reward.

DC01: Identification of potential environmental sources of airborne pathogens: Description of an early-detection model

Jeroen Van Leuken[1,2], Arie Havelaar[1,2], Wim Van der Hoek[2], Georgia Ladbury[3], Volker Hackert[4], Arno Swart[2]

1. *Institute for Risk Assessment Sciences (IRAS), Utrecht University, Utrecht, The Netherlands.*
2. *Centre for Infectious Disease Control (CIb), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.*
3. *University of Glasgow, Glasgow, United Kingdom.*
4. *Municipal Health Service Zuid---Limburg, Sittard---Geleen, The Netherlands.*

Source identification in areas with outbreaks of airborne pathogens is often time-consuming and expensive. We developed a model to identify the most likely location of sources of airborne pathogens.

As a case study, we retrospectively analysed three Q fever outbreaks in the Netherlands in 2009, with suspected exposure from a single large dairy goat farm. Model input consists only of residences of cases, day of first clinical symptoms, and human population density data. We defined a spatial grid and fitted the incidence- distance relation for each grid point to an exponentially declining function. For any grid point significant at the 95% confidence level, we calculated a measure of risk. For validation, we used results from systematic bulk tank milk sampling at large (i.e. >50 goats and/or sheep) dairy farms, and non-systematic vaginal swab sampling at several non-dairy goat/sheep farms.

Hotspots – areas most likely to contain the actual source - are identified in early stages of the outbreaks using only 2 – 10% of the case notifications. Distances between the hotspots and suspected goat farms vary from 300 – 1500 m. By ranking all large dairy farms, the suspected goat farms are listed as the most likely source.

Our model identifies the most likely location of sources in an airborne pathogen outbreak area, even in early stages, thereby reducing the number of potential sources to be investigated and allowing rapid implementation of interventions to limit the number of human infections and to reduce the risk of between-source transmission.

DC02*: Within-herd transmission model for Q fever in Dutch dairy goats and ranking of control measures

D.M. Bontje[1], J.A. Backer[1], L. Hogerwerf[2], H.I.J. Roest[1], M. van Asseldonk[2], R. Bergevoet[2], A.A. de Koeijer[1], H.J.W. van Roermund[1]

1. *Central Veterinary Institute (CVI) of Wageningen UR, Lelystad, The Netherlands*
2. *Agricultural Research Institute (LEI) of Wageningen UR, Wageningen, The Netherlands*

In the Netherlands *Coxiella burnetii* infected four thousand humans with Q-fever in the period 2007-2011, leading to 24 confirmed deaths. This human Q fever problem was related to dairy goat farming and exposure was likely from dung dust particles from commercial dairy goats herds. Human exposure was successfully reduced by culling pregnant goats from test-positive herds followed by preventive vaccination of whole goat herds, leading to economic damage, loss of animals and crippling of commercial enterprises.

Based on the biology of Q fever and the situation of commercial Dutch dairy goat herds, a within-herd transmission model was derived, with the aim of analysing the efficacy and efficiency of control measures. Using literature and expert opinion we formulated a flow-chart which summarizes and represents current knowledge on the biology and pathogenicity of *Coxiella burnetii* in goats, leading to a stochastic simulation model. Simulated control measures include: 1) none, 2) preventive yearly vaccination and five reactive strategies, 3) vaccination after bulk tank milk (BTM) positive, 4) breeding ban after BTM positive, 5) *Search&Destroy* after BTM positive, 6) culling of pregnant goats after abortion storms and 7) vaccination after abortion storms.

We found that only strategies which involve vaccination are serious options for Q fever control. When taking social economic aspects including loss of human health into account, preventive vaccination is the best strategy to remove the disease from a herd, followed by "Vaccination after abortion storm" and "Vaccination after BTM positive".

DC03: Syndrome surveillance among families with young children: a new method for infection-transmission surveillance?

M. Koningstein[1], R. Zuidema[1], R. Enserink[1], E. Duizer[2], T. Kortbeek[2], M.C. Scholts[3], J Roelfsema[2], M. Koopmans[2], A.M.D. Kooistra-Smid[3,4], W. v Pelt[1]

1. *Department of Epidemiology, National Institute for Public Health and the Environment (RIVM), Bilthoven*
2. *Department of Infection Detection and Screening, RIVM, Bilthoven*
3. *Department of Research and Development, Laboratory for Infectious Diseases (LvI), Groningen*
4. *Dept. of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands*

Upper-respiratory (URI) and gastrointestinal illness (GII) are common among young children (0-4 years). Children attending daycare centres (dcc) experience 1.5-3 times more infectious episodes than children who do not attend a dcc. Consequently, these children are more likely to be treated with antibiotics. Because of the mechanism of selection pressure, these children are more likely to carry antimicrobial resistant pathogens and ESBLs. Due to the close contact between young children and their caregivers, these children might be a source of antimicrobial resistant pathogens for adults and thus for the general population.

We aim to determine the contribution of young children in the transmission of illness due to GII and URI to the general population, and the role of dcc therein. The microbiological component will focus on the transmission of enteropathogenic pathogens, antimicrobial resistance and ESBLs.

Data is gathered through monthly, web-based self-reported disease-syndrome surveillance in combination with feces sampling among (healthy) caregiver-child couples.

Monthly, 2000 questionnaires are sent, of which on average 20% are filled out and returned for both child and one caregiver. On the question whether parents would also be willing to send a fecal sample of themselves and their child to the laboratory 35% (141 couples per month) answered positively. As of yet, feces sampling has not started, it will start in April.
From the questionnaires, we found significant differences in the self-reported symptoms for GII and URI between dcc and non-dcc attending children and their parents.

DC04*: Genomic variation of *Salmonella* serovar Infantis

Eva Litrup[1], Kristoffer Kiil[1], Zhemin Zhou[2], Gitte Sørensen[3], David Guttman[4], Mia Torpdahl[1], Mark Achtman[2], Eva Møller Nielsen[1]

1. *Statens Serum Institut, Copenhagen, Denmark*
2. *University College Cork, Cork, Ireland*
3. *National Food Institute, Technical University of Denmark, Lyngby, Denmark*
4. *University of Toronto, Toronto, Canada.*

Salmonella enterica serovar Infantis is one of the top ten serovars causing human disease in Europe, and it is a frequent serovar detected in broilers and layers. Infantis strains often display resistance to several antibiotics.

A worldwide collection of 79 *S.* Infantis strains were selected to represent the known diversity. We detected 10 sequence types (ST) in the Infantis strains. The main cluster consists of 66 strains with ST32 and five variants of this main ST. Four strains formed a non-related group based on their STs, and these strains were isolated in, or were travel-related cases to Africa and Asia. An additional two *S.* Infantis strains were non-related to the main cluster by MLST. When evaluating the O-antigen and flagella genes, the non-related strains showed minor differences to the main cluster. These results suggest that *S.* Infantis is a polyphyletic serovar.

The strains were sequenced, and based on SNP distance the majority of strains fell into a main cluster. The remaining strains, that were un-related by MLST, were located on very long branches and were as such unrelated to the main cluster. The main cluster was divided into two groups. The larger group (n=59) contained a cluster that was significantly enriched with resistant strains. The smaller group (n=12) consisted mainly of strains isolated from animals and feed. Preliminary results suggest a different bacteriophage reservoir in the small group consisting of veterinary strains.

Detection of a veterinary group raises the question whether there is a veterinary adapted clone of *S.* Infantis.

DC05: Role of plasmids in the increased virulence of Dutch *Coxiella burnetii* goat strains

Runa Kuley[1,2], Hilde Smith[1], Frank Harders[1], Jerry Wells[2], Mari Smits[1,2], Dimitrios Frangoulidis[3], Hendrik Jan Roest[1] and Alex Bossers[1]

1. *Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands*
2. *Wageningen University, Wageningen, The Netherlands*
3. *Bundeswehr Institute of Microbiology, Munich, Germany*

Coxiella burnetii (Cb), the etiological zoonotic agent of "Q fever" in humans and animals, is an obligate intercellular BSL-3 bacterial pathogen. During 2007-2010 an unprecedented Q fever epidemic occurred in The Netherlands where most likely over 40,000 people were infected. Molecular characterisation of the Dutch outbreak isolate is essential for (molecular) epidemiological and vaccine development studies. Cb always carries a plasmid or plasmid-related DNA sequences in the chromosome, implying a critical function for some core plasmid genes. It is hypothesised that plasmid-related DNA sequences in Dutch Cb isolates could encode essential virulence modulators.

To identify, isolate and characterise plasmid (derived) sequences in Dutch Cb isolates. Assess virulence differences in Cb isolates transformed with plasmid/chromosomal elements of Dutch strains using a mouse virulence bioassay.

The bacterial plasmids were isolated from Dutch Cb isolates. In all isolates we found plasmid or plasmid-derived sequences which were further identified by NGS sequencing. We have demonstrated that Dutch Cb derived DNA sequences can increase the virulence of less virulent strains after transformation of plasmid or plasmid-derived sequences in a mouse virulence bioassay.

Plasmid or plasmid-derived sequences from Dutch goat isolates were able to increase virulence, indicating that these sequences may encode key virulence factors/modulators resulting in increased fitness of Cb. Further investigation towards the exact nature of these plasmid/plasmid-derived sequences in these Dutch strains, as well as their interaction with components from the chromosome, is essential to determine key virulence factors, direct treatments, transmission prevention systems, and vaccine development.

DC06: *S. aureus* growth in cheese produced with raw goat milk

Alvaro Mendonça[1,3], Manuel Machado[2], Alcina Tavares[2], Raimundo Maurício[3], Manuel Cardoso[3]

1. *CIMO – Centro de Investigacão de Montanha. Quinta de Sta Apolónia, Apart 1172, 5301-855 Braganca – Portugal.*
2. *Instituto Nacional de Investigação em Agricultura e Veterinária.*
3. *Escola Superior Agrária de Braganca. Quinta de Sta Apolónia, Apart 1172, 5301-855 Braganca – Portugal.*

Traditional high-quality goat cheeses (PDO) are produced with raw milk in many Mediterranean countries; the flavor from these cheeses is particularly different from those produced with pasteurised milk and is very popular amongst consumers. It is a very important industry, from an economic point of view.

However, there are several microbiological hazards in raw milk that can be dangerous for consumer's health, namely *Staphylococcus aureus,* associated with goat mastitis. Our objective is to evaluate the potential problem of this pathogen in goat cheese.

For this purpose, raw milk was inoculated with a known amount of *S. aureus* cfu/ml of milk, and used for cheese production. The specific strain of *S. aureus* selected for study was isolated from a commercial flock. During 4 weeks ripening, the total cfu/g cheese and staph toxin was evaluated weekly. Cheeses were ripened at 10º Celsius.

The work is still in progress, but we were able to show that *S. aureus* is able to survive the ripening process. We do not yet have results about toxin production, because cheese samples were frozen for ulterior global processing. We intend to have final results by April, 20th.

This work focuses on the relationship between animal health and biological risks for consumers.

DC07: Neurotoxin Gene Profiling of *Clostridium botulinum* Types C and D Native to Different Countries within Europe

Cedric Woudstra, Patrick Fach

1. *Laboratory for Food Safety, French Agency for Food, Environmental and Occupational Health Safety (ANSES), Maisons-Alfort, France*

Clostridium botulinum types C and D and their mosaic variants C-D and D-C are associated with avian and mammalian botulism. This study reports on the development of low-density macroarrays based on the GeneDisc cycler platform (Pall-Gene-Disc Technologies) applied to the simultaneous detection of the *C. botulinum* subtypes C, C-D, D, and D-C. The limit of detection of the PCR assays was 38 fg of total DNA, corresponding to 15 genome copies. Artificially contaminated samples of cecum showed a limit of detection below 50 spores/g. The tests were performed with a large variety of bacterial strains, including *C. botulinum* types C (n_12), C-D (n_29), D (n_5), and D-C (n_10), other botulinum neurotoxin (BoNT)-producing *Clostridium* strains (n_20), non-BoNT-producing clostridia (n_20), and other bacterial species (n_23), and showed a high specificity.

These PCR assays were compared to previously published real-time PCRs for the detection of *C. botulinum* in 292 samples collected from cases of botulism events in four European regions. The majority of the samples originated from wild birds (n_108), poultry (n_60), and bovines (n_56). Among the 292 samples, 144 were positive for either bont/C-D or bont/D-C gene by using the GeneDisc arrays. The reliability of the results tallied to 97.94%. Interestingly, only BoNT mosaics, types C-D and D-C, were found in naturally contaminated samples, whatever their animal origin and their geographical location. Further investigations should now be performed in order to check that mosaic types dominate in Europe and that acquisition of mosaic types helps in survival or adaptation to particular niche.

DC08: Protecting animal and human health: Investigating correlates of vaccine protection for the lyssaviruses

Jennifer S Evans[1,2], Edward Wright[3], Andrew J Easton[2], Anthony R Fooks[1], Ashley C Banyard[1]

1. *Animal Health and Veterinary Laboratories Agency, Woodham Lane, Weybridge, Surrey, KT15 3NB, United Kingdom*
2. *School of Life Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom*
3. *University of Westminster, 115 New Cavendish Street, London, W1W 6UW, United Kingdom*

Rabies, the archetypal lyssavirus, is one of the most feared viruses known to man and causes >50,000 deaths per year. Other members of the lyssavirus genus cause clinical disease consistent with rabies. The lyssavirus glycoprotein is the sole target for virus neutralising antibodies and several amino acid epitopes have been linked to virus neutralisation.

Lyssaviruses are segregated into phylogroups that indicate the level of protection afforded by current vaccines. It is generally accepted that an antibody response to rabies vaccines affords protection against all viruses that are categorised into phylogroup I. However, this antibody response does not protect against lyssavirus species within phylogroups II and III. Indeed, experimental data has shown that the antibody repertoire induced by rabies virus vaccines is completely unable to neutralise viruses in these phylogroups.

In this study we have generated lentivirus pseudotypes containing chimeric lyssavirus glycoproteins that have had their antigenic sites swapped between phylogroup I and II viruses. With these we have assessed neutralisation of both wildtype and chimeric pseudotype particles with both phylogroup I and phylogroup II specific hyperimmune sera. We have used chimeric pseudotype viruses to analyse the role of defined antigenic domains in development of phylogroup specific immune responses.

DC09: Spread of hepatitis E virus from pig slurry to the water environment

Jesper Schak Krog[1],Anita Forslund[2], Solvej Østergaard Breum[1], Lars Erik Larsen[1], Anders Dalsgaard[2], Anna Charlotte Schultz[3]

1. *National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1790 Copenhagen V, Denmark.*
2. *Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen*
3. *Division of Microbiology and Risk Assessment, National Food Institute, Technical University of Denmark*

The objective of this study was to examine if hepatitis E virus (HEV) could be transported through the soil and into the drainage system, thus presenting a risk for virus transmission to wildlife and humans.

Slurry from a Danish pig farm was spread on a tile-drained field of loamy soil. Water that arrived at the drainage system located 1 m below surface was collected over a time period of 4 months. Samples were collected on a weekly basis and whenever water flow in the drainage system exceeded a certain threshold in connection with heavy rain events. In addition, samples of water were collected from wells located along the field and groundwater. Archived mussels from different regions in Denmark were tested for HEV, too. Samples were concentrated, extracted, purified and subsequently tested for HEV by real-time RT-PCR.

Water samples representing a total of 14 events were included. HEV was detected in the first event following spread of pig slurry. In agreement with this finding, the weekly sample of this period also tested positive. HEV was not found in any of the subsequent water samples. None of the 70 blue mussel samples, which mainly originated from fjords, tested positive.

HEV is regarded as a zoonotic virus with pigs as the primary reservoir. The pathway to humans and other mammals is unclear. Here we show that under Danish conditions, spread of pig slurry can cause viral contamination of water reservoirs, making HEV accessible to the human population and wildlife.

DC10: The usefulness of FTA® filter papers for the molecular detection of avian influenza viruses with zoonotic potential

Zenon Minta, Krzysztof Wyrostek, Michał Jóźwiak, Monika Olszewska, **Krzysztof Śmietanka**

National Veterinary Research Institute, Department of Poultry Diseases, Al. Partyzantów 57, 24-100 Puławy, Poland

Avian influenza viruses (AIV) of H5N1, H9N2 and a variety of H7 subtypes (including most recent H7N9 in China) may have zoonotic potential. The successful detection of an influenza virus depends upon the prompt collection of high-quality samples and their rapid transportation to the laboratory, preferably with cold-chain maintenance. In this study we investigated the utility of FTA® cards as an alternative sampling device for the detection of H5N1 (highly pathogenic) and H7N1 (low pathogenic) AIV.

The viruses lost infectiveness within 1 hour after adsorption to the FTA® card at room temperature. The minimal detectable viral titer obtained in our study and measured in real-time RT-PCR was equivalent to $10^{3,0}$ EID_{50}/ml. The FTA®-adsorbed viral RNA was detectable for 150 days at room temperature, -20°C and -70°C, irrespective of the virus subtype. However, noticeable differences in the viral RNA loads were observed indicating that deep freezing creates favourable conditions for long-term storage. The PCR product obtained as a result of RT-PCR with the RNA extracted from FTA® cards incubated 90 days at room temperature was successfully sequenced. The organ samples collected at 3 and 4 days post infection from SPF chickens experimentally infected with H5N1 HPAIV were used to make smears on FTA® cards and the real time RT-PCR showed very high viral loads in all tested samples. The FTA® cards appear to be a safe and effective alternative sampling method for diagnosis of AI.

DC11*: One health – One flu: Surveillance in pigs and mink has revealed extensive exchange of influenza A virus genes and viruses among animals and humans

Solvej Ø. Breum, Charlotte K. Hjulsager, Ramona Trebbien, Kristina Fobian, **Lars E. Larsen**

National Veterinary Institute, Technical University of Denmark, Bülowsvej 27, 1870 Frederiksberg C, Denmark.

Until the H1N1 pandemic influenza A virus emerged in humans in 2009, surveillance programmes for influenza A viruses in non-avian animal species were sparse and unsystematic. Since 2009, passive surveillance programmes focusing on detection and characterisation of influenza A viruses in swine have been launched in several countries. In Europe the results of these surveillance programmes have been coordinated by the EU FP7 project European Surveillance Network for Influenza in Pigs (ESNIP3), coordinated by AHVLA, UK. During the years 2009-2013, the surveillance programmes in Denmark have resulted in detection of new influenza A virus reassortants in swine. Several of these new viruses contain N genes previously detected in human influenza A viruses. Other European countries and USA have reported similar results.

Concurrently, the passive surveillance in mink for influenza A virus revealed a new influenza virus containing the important H and N genes of human H3N2 virus origin. This new virus was responsible for severe respiratory disease and high mortality in 25 Danish mink farms in 2009. Furthermore, in 2010 and 2011 the H1N1 pandemic influenza A virus was detected in diseased Danish mink. The detection of "human pathogenic" influenza A viruses or genes in animal species emphasises that influenza A virus circulating in all animal species should be considered a potential zoonotic threat and regarded as the most important "one health pathogen".

DC12: Antimicrobial resistant *Escherichia coli* on lettuce fertilised with poultry manure and irrigated with low quality water in Kumasi, Ghana

Karin Malmros[1], Bernard Keraita[2], Kwaku Peprah Owusu[3], Kwasi Obiri-Danso[3], Anders Dalsgaard[1]

1. *Department of Veterinary Disease Biology, University of Copenhagen*
2. *Department of International Health, Immunology and Microbiology, University of Copenhagen*
3. *Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology*

Outbreaks of gastroenteritis due to ingestion of contaminated vegetables are important human health problems, especially in many low- and middle-income countries. The likelihood of contamination with enteric pathogens is often indicated by quantification of *Escherichia coli* which also is used as an indicator to monitor antibiotic resistance. This study was conducted on an urban vegetable farm in Ghana. The aim was to assess the occurrence of antimicrobial resistant *E. coli* on lettuce irrigated with tap water ($<10^2$ *E. coli*/100ml) or shallow well water ($\sim10^3$ *E. coli*/100ml) and fertilised with poultry manure or commercial NPK fertiliser. The four treatment combinations were tested using a systematic 4x4 Latin square design.

E. coli with potential extended spectrum beta lactamases (ESBL) was enumerated directly on 3M™ Petrifilm™ Select *E. coli* count plates containing cefotaxime (0.25 mg/L). The results did not demonstrate a significant difference in the occurrence of *E. coli* with potential ESBLs, but a contamination level of 0.25-1.4 cefotaxime resistant *E. coli*/g lettuce was detected irrespective of what treatment the lettuce had been subjected to. The overall concentration of *E. coli* was on average 10^2-10^3 cfu/g lettuce, regardless of the treatment applied. These results suggest that the majority of the *E. coli* originated from sources other than the irrigation water and the fertiliser, possibly from the soil. The exact origin of the *E. coli* needs to be identified in order to assess potential food safety risks associated with irrigation with low quality water and application of poultry manure when culturing lettuce.

DC13: Bead-based DNA assay for the detection of *Streptococcus suis* in tonsillar specimens of pigs

Fimme Jan van der Wal[1], Henk Wisselink[1] , Jan Bergervoet[2], Marjanne de Weerdt[2], René Achterberg[1]

1. *Central Veterinary Institute of Wageningen UR, Lelystad, the Netherlands*
2. *Plant Research International of Wageningen UR, Wageningen, the Netherlands*

Streptococcus suis is an important swine pathogen in nearly all countries with an extensive pig industry. In pigs it is associated with meningitis, arthritis, endocarditis, septicemia, pneumonia and sudden death. In certain parts of Asia *S. suis* is a common cause of bacterial meningitis in humans. Attempts to control *S. suis* in pig farming are hampered by the lack of sensitive diagnostic tools and effective vaccines.

To provide a basis for adequate control measures, a bead-based suspension array was developed that targets six genes using multiplex PCR followed by target specific primer extension (TSPE) and subsequent hybridisation to beads. Assays were designed using AlleleID and PrimerPlex on consensus sequences of four serotype specific capsular polysaccharide (cps) genes (cps1I, cps2J, cps7H, cps9H), the extracellular virulence factor EF (epf) and a general S. suis marker (glutamate dehydrogenase; gdh). The proof-of-principle of the assay was demonstrated with chromosomal DNA of reference strains: each PCR was capable of producing the desired amplicon, also when performed in a sixplex reaction. Upon hybridisation of the TSPE products, all multiplex PCR products produced signals on the appropriate beads.

This assay will be evaluated with field samples, using DNA isolated from tonsillar specimens of pigs. Ultimately, this expandable assay may be applicable for studying epidemiology and transmission, and could contribute to efforts aimed at control and eradication of *S. suis* in pig farming, thereby contributing to human health.

DC14: Antimicrobial susceptibility of *Bordetella avium, Ornithobacterium rhinotracheale* and *Riemerella anatipestifer* strains from wild and domesticated birds in Hungary

Réka Szabó[1], Éva Gyuris[2], Enikő Wehmann[1], Tibor Magyar[1]

1. *Institute for Veterinary Medical Research, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary*
2. *Veterinary Diagnostic Directorate, Central Agricultural Office, Budapest, Hungary*

The emergence of multi-drug resistance is now becoming one of the major medical and veterinary concerns. Animals are considered potential reservoirs of multidrug-resistant Gram-negative organisms. The indiscriminate overuse of antibiotics in veterinary medicine has contributed to the selection of resistant pathogens. At the same time, the food production industry increasingly demands the minimal use of antibiotics in food production animals. Thus, up to date data on the antimicrobial susceptibility of bacterial isolates is essential for a sensible and effective use of antibiotics in practice.

Respiratory tract infections are causing considerable economic loss in the poultry industry around the world. *Bordetella avium*, *Ornithobacterium rhinotracheale* and *Riemerella anatipestifer* are among the several pathogens associated with respiratory disease. Antimicrobial susceptibility of 20 *B. avium*, 38 *O. rhinotracheale* and 55 *R. anatipestifer* strains were determined by Kirby-Bauer disk diffusion method. Most strains were resistant to nalidixic acid, lincomycin, sulphamethoxazole trimethoprim and sulphonamides, and were susceptible to ampicillin, amoxicillin and doxycycline. As expected, strains isolated from wild birds were susceptible to considerably more antibiotics than strains from domesticated poultry species. Recent isolates showed greater resistance than strains from the 1980s, indicating the spread of resistance in these bacterium species. No plasmids were isolated from *O. rhinotracheale* strains, while 50 % of the examined *R. anatipestifer* strains yielded a ~9000 bp sized plasmid.

DC15: T*aenia solium* cysticercosis – an emerging foodborne zoonosis in sub-Saharan Africa

Maria Vang Johansen, Faustin Lekule,Helena Ngowi, Yunus Assane, Eliakunda Kimbi, Alberto Pondja, Pascal Magnussen, Helena Mejer, Stig Milan Thamsborg

Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen

Pig-keeping and pork consumption have increased significantly in eastern and southern Africa (ESA) during the past decade. A high and increasing prevalence of epilepsy in ESA, without a clear etiology, and an increase in cases of porcine cysticercosis have been noted in the region.

Two Danida-funded projects have addressed the problem, first by assessing the prevalence, risks and impacts of *T. solium* taeniosis/cysticercosis in both humans and pigs in Mozambique and Tanzania from 2006-2009, and, through an on-going project, by trying to develop sustainable solutions for control of the disease. The study areas include Tete province, Western Mozambique and Mbeya region, southern Tanzania. The prevalence of *T. solium* cysticercosis in the areas was found to be between 31-35% in pigs and 15-18% in humans based on an Ag-ELISA. In addition 45% of the human population was found to be Ab-positive for cysticercosis. Among a subgroup of the participants in Mozambique, 72% (77/107 Ag-positive) compared to 18% (8/44 Ag-negative) were having abnormal CT scans suggestive of neurocysticercosis. Epilepsy was, in both countries, very common and strongly associated with stigmatisation. Risk factors for *T. solium* infections included poor pig husbandry practices especially free ranging pigs, open defecation, age of pigs, pork cooking practices, lack of meat inspection, and lack of knowledge regarding transmission of the disease. The on-going project focuses on health education and proper pig management as means to control not only *T. solium* cysticercosis, but also African swine fever, another serious constraint for improving the livelihood of smallholder pig producers in the region.

DC16: HDP2-PCR diagnosis of the complex Taeniosis/Cysticercosis

María Flores[1], Carolina Hurtado[1], Yamileth Monje[1], Luis Miguel González[1], **Maria J. Perteguer[1]**, Teresa Gárate[1].

1. *Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220, Madrid, Spain.*

Molecular diagnosis of parasitic diseases has proved to be an essential tool in the rapid, specific and sensitive detection of protozoa and helminthic infections (Mejia *et al.* Am J Trop Med Hyg. 2013). In this sense, we used a repetitive DNA fragment, HDP2, to approach the taeniosis/cysticercosis diagnosis (González LM *et al.*Trans R Soc Trop Med Hyg. 2002;96 Suppl 1:S243). HDP2 was characterised in Taenia saginata, *Taenia asiatica* and *Taenia solium* (González *et al.* Parasit Vectors. 2010;3:51). Based on both screening of the taeniid genomic libraries with specific HDP2 probes and particular PCR amplification from the parasitic genomic DNAs, HDP2 was identified as non-transcribed ribosomal DNA (NTS). Furthermore, HDP2 showed dissimilar structure according to the distinct three species, which could allow the differential species-specific identification of each human taeniid. Considering the characteristics observed in the repetitive DNA fragments, PCRs were designed that permitted the diagnosis of human intestinal taeniosis, animal cysticercosis and human neurocysticercosis (mainly extra-parenchymal cases). These data will be discussed in comparison to other diagnostic molecular markers.

The work was supported by RETIC-RICET (RD12/0018/011) and FIS (PI10/01627)

DC17: Molecular identification of *Anisakis simplex* species

Pamela Campioli, Teresa Gárate, **Maria J. Perteguer**

Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220, Madrid, Spain

Nearly 20,000 cases of anisakiosis are reported annually, most of them in Japan and in coastal areas of Europe (Hochber *et al*., 2010). Spain is one of the regions with the highest human anisakiosis incidence; more recently the number of both human cases and parasite fish burden has increased (Daschner and Cuellar, 2012). This work proposed the development of molecular markers to allow the identification of the causative agents of anisakiosis. It is accepted that *Anisakis simplex sensu lato*, from all the anisakid genotypes, is the most frequently species involved in human disease considering that the complex *A. simplex* comprises sibling species. Several PCR techniques were standardised using ribosomal (18S, ITS-1, 5,8S, ITS-2 and 28S genes: PCR-NC5/NC2; PCR-CQ3F/CQ3R; PCR-CQ6F/NC2; PCR-CQ1F/CQ1R; PCR-CQ4/CQ4R) and mitochondrial (Cox 2 gene, PCR-211/210) markers.

The use of the above techniques confirmed the presence of different anisakid species in a single fish, *Hysterothylacium aduncum* and *Anisakis simplex sensu stricto*. Further, larvae from the sibling species *A. simplex* (*s.s.*), *Anisakis pegreffi* and the hybrid *A. simplex* (*s.s.*)-*A. pegreffi* were identified in an infected fish from North Atlantic ocean. All these data confirmed the existence of a sympatric area for these species and the existence of the hybrid in the Atlantic coasts.

DC18: Evaluation of pre-PCR processing approaches for enumeration of *Salmonella enterica* in naturally contaminated animal feed

Charlotta Löfström[1], Jenny Schelin[2], Gunnar Andersson[3], Håkan Vigre[1], Börje Norling[4], Per Häggblom[3], Jeffrey Hoorfar[1] & Peter Rådström[2]

1. *National Food Institute, Technical University of Denmark, Søborg, Denmark*
2. *Applied Microbiology, Department of Chemistry, Faculty of Engineering, Lund University, Lund, Sweden*
3. *National Veterinary Institute (SVA), Department of Chemistry, Environment and Feed hygiene, Uppsala, Sweden*
4. *Quintessence Research AB, Ramhäll 119, Alunda, Sweden*

To facilitate quantitative microbial risk assessment (QMRA) in the feed production chain, there is a need to generate data on the numbers of *Salmonella* in naturally contaminated feed. The objective of the present study was to evaluate three pre-PCR processing strategies for the detection and/or quantification of *Salmonella* in naturally contaminated soy bean meal; (i) flotation-qPCR, a non-destructive sample preparation method for direct enumeration of intact Salmonella cells prior to quantitative PCR (qPCR), (ii) MPN-PCR, a modified most probable number method combined with real-time PCR and (iii) qualitative culture enrichment PCR. Fifteen naturally contaminated soya bean meal samples from one feed lot were analysed in parallel with the three pre-PCR processing strategies, using 2.5, 50 and 25 g of feed, respectively, prior to a standardised, real-time PCR assay specific for *Salmonella*.

Results show that *Salmonella* was qualitatively detected in 6, 15 and 9 bags out of 15 for flotation-qPCR, MPN-PCR, and culture enrichment PCR, respectively. Enumeration resulted in values of 1.8×10^{2}-7.8×10^{3} CFU/g (flotation-qPCR) and 0.024 to >5.2 MPN/g (MPN-PCR). The differences in results obtained with the three techniques could be due to the presence of non-culturable *Salmonella* and/or a heterogeneous distribution of *Salmonella* in the feed. In conclusion, the evaluated methods provide different possibilities to assess not only the prevalence of *Salmonella* in feed, but also the numbers of culturable, as well as non-culturable cells. These methods can be applied to generate data to support more accurate QMRA for *Salmonella* in the feed chain, relevant in a One Health approach.

DC19: Pooling of cattle fecal samples for detection of *Salmonella*

Elina Lahti, Lennart Melin

National Veterinary Institute (SVA), Uppsala, Sweden

In Sweden a *Salmonella* control program for the whole production chain has been in place since more than 50 years. When *Salmonella* is detected in a food-producing animal, the herd is sampled and analysed according to ISO 6579:2002, Annex D. Pooling is applied in order to decrease costs. The purpose of this study was to evaluate pooling before pre-enrichment and after pre-enrichment as well as sample sizes 5 and 25 g.

Fecal material from one animal was inoculated with three levels of *Salmonella* Typhimurium or Dublin: 10 cfu/25 g, 100 cfu/25 g and 1000 cfu/25 g. All the inoculated samples were analysed both individually and after pooling. Altogether six replicates were used in each trial. A negative control was included.

S. Typhimurium could be detected from all subsamples irrespective of the pooling method or sample size. *S.* Dublin could be detected from all individual samples except for one sample of 5 g. Also, *S.* Dublin could be detected from all subsamples incoulated with 100 or 1000 cfu. However, at 10 cfu/25 g, *S.* Dublin was detected in 8/12 subsamples of 25 g pooled before pre-enrichment and from 11/12 subsamples of 5 g pooled after pre-enrihment.

The study shows that for *S.* Typhimurium pooling of five cattle fecal samples before pre-enrichment is as sensitive as analysing individual samples. However, at a low level, the sensitivity of the method for *S.* Dublin decreases.

DC20: Validation and adoption of quantitative PCR methods for the diagnosis of Q fever

Rousset, E.[1], Prigent, M.[1] De Cremoux, R.[2], Sidi-Boumedine, K.[1]

1. *Anses, Sophia-Antipolis, France*
2. *Institut de l'Elevage, Albi, France*

In France, Q fever is a major animal and public health concern. For a better assessment and management of risks, a syndromic monitoring is being implemented in ruminant livestock, constituting the primary reservoir of the disease. The main objective is the production of reliable and comparable data through the network of veterinary laboratories involved in the analyses. To achieve this, the standardisation of PCR methods for this plan was conducted following the directives of the new AFNOR norm on PCR in Animal Health (XP-U47-600-1 and -2). The process was coordinated by the French Q fever National Reference Laboratory (NRL). First, the required reference materials were prepared (purified titrated bacteria, bacterial genomic DNA). Two PCR kit suppliers and a departmental laboratory, as method developers, have performed the tests in accordance with the norm, to validate their methods. The NRL has formalised a common procedure, as well as the performance requirements to meet the needs and expectations for the monitoring plan (quantitative PCR, extended range on 5 $\log_{10}$ including diagnostic thresholds and the level of 10^6 bacteria per swab, accuracy of results). Therefore, developers have determined the performance characteristics of their methods.

Also in this normative framework, in the case of commercial validated methods (PCR kit suppliers), a final step was required before routine implementation. Thus, the NRL organised the adoption step for departmental laboratories, as users, to ensure the achievement of the expected performance in terms of limit of detection and accuracy of quantification.

To our knowledge, this is the first attempt of a collaborative approach to adopt a validated quantitative PCR method involving veterinary departmental laboratories, in France. The results of this adoption step of quantitative PCR allowed the control of the degree of performances and the checking of the consistency of results both at the intra- and inter-laboratory levels.

DC21: Development of rapid point of care tests for the detection of bacteria implicated in surgical site infections

Onyinye Diribe[1, 2], Sarah North[1], Noel Fitzpatrick[3], Lisa Roberts[2], Jason Sawyer[1], Manal AbuOun[1], Roberto M. La Ragione[1, 2]

1. *Animal Heath and Veterinary Laboratories Agency, Weybridge, Surrey, KT15 3NB, UK.*
2. *Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, GU2 7XH, UK.*
3. *Fitzpatrick Referrals, Halfway Lane, Godalming, Surrey, GU7 2QQ, UK.*

Surgical site infections (SSI) remain a problem in both human and veterinary medicine. SSIs can have far-reaching implications for surgery outcomes. Therefore, it is essential that the causative agent(s) of an SSI is (are) rapidly identified to aid targeted treatment. Application of molecular techniques such as PCR in private veterinary clinics has been limited by the instrumentation, cost and technical expertise they require. The work described here aimed to develop rapid molecular diagnostic tests requiring minimal sample preparation and instrumentation, for elucidation of organisms commonly implicated in SSIs.

Loop mediated Isothermal Amplification (LAMP) was used as the basis of the rapid diagnostic tests. A set of six organism-specific were designed and tested against characterised clinical and control isolates. The assays were subsequently modified to allow incorporation and detection using a lateral flow device (LFD).

We developed assays targeting *Staphylococcus aureus, S. epidermidis, S. haemolyticus, Pseudomonas aeruginosa*, *Escherichia coli, Enterococcus faecalis* & *faecium*, and *Staphylococcus pseudintermedius*. On a panel of 27 clinically relevant bacteria, the assays demonstrated 100% specificity, capable of detecting less than 4 gene copies, as well as being robust enough to be used with lysate prepared directly from a clinical swab, without purification or cleaning. Detection was determined within 15 minutes in all the assays.

LAMP is a viable alternative to conventional PCR and culture-for the detection of bacteria, and is amenable to use for rapid and cost efficient diagnostic pen-side tests in routine diagnosis.

HP01*: The role of red deer (*Cervus elaphus*) as reservoir host for *anaplasma phagocytophilum*

Kristin Sæbø Pettersen[1], Richard John Birtles[2], Kevin John Bown[2], Erik Georg Granquist[3] and Snorre Stuen[4]

1 *Norwegian Veterinary Institute, Oslo, Norway.*
2 *School of Environment and Life Sciences, University of Salford, Salford, United Kingdom*
3 *Norwegian School of Veterinary Science, Department of Production Animal Clinical Sciences, Oslo, Norway.*
4 *Norwegian School of Veterinary Science, Department of Production Animal Clinical Sciences, Sandnes, Norway.*

Co-existence of wildlife and their vectors, domestic animals and humans, may become one of the main sources for several emerging foodborne and non-foodborne zoonoses. Effects exerted by changes to climate, demography and agriculture upon ecosystems, are future challenges to be met by the "bio-economy" approach in order to ensure and secure sustainable animal-based food production. *Anaplasma phagocytophilum* is an intracellular pathogen, transmitted by ixodid ticks to domestic ruminants, wild game and humans in Europe, to cause primary illness, immune suppression, secondary infections, severe direct and indirect economic losses and animal welfare implications. The study of natural transmission cycles by unravelling molecular elements of the host-pathogen-vector triangle may improve food safety, animal welfare and public health in scope of the one health, one medicine approach.

In this study, we examined the inter-species strain relationships of *A. phagocytophilum*, isolated from deer and sheep. By molecular characterisation of the *16S rDNA* and the gene encoding the major surface protein 4 (MSP4), a protein believed to be important for host cell infection, we investigated whether red deer and sheep belong to the same natural transmission cycle.

The results indicate a certain host predilection among homologous *16S rDNA*, shown by differentiation at the *msp4* level. The current belief that red deer and sheep are part of the same endemic *A. phagocytophilum* transmission cycle needs reconsideration and further elucidation based on the current results.

HP02: Understanding the fitness burden of extended-spectrum β-lactamase harbouring plasmids in avian pathogenic *E. coli*

Mishaal Alanazi, Jenny M. Ritchie, Jane. Newcombe and Roberto M. La Ragione

School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, UK

Avian colibacillosis is an economically important infectious disease of domestic poultry. The aetiological agent responsible for colibacillosis is *Escherichia coli*. Recently, APEC isolates with plasmid-encoded extended-spectrum β-lactamase (ESBL) resistance have been reported. ESBL plasmids are increasingly prevalent within animal and human bacterial isolates, yet little is known about the effect of ESBL plasmid carriage on the host bacterium. Thus, the aim of this study was to investigate the impact of ESBL plasmid carriage on bacterial fitness.
Three ESBL plasmids were extracted from unrelated APEC strains and separately introduced into non-ESBL plasmid-carrying APEC and *E. coli* K12, respectively. The strains were then characterised using molecular and phenotypic assays. The rate of ESBL plasmid transmission was also investigated under a range of pH conditions.

The studies confirmed that all the ESBL plasmids belonged to the same Inc type (I1-IY), were group 1 and 148Kb in size. ESBL plasmid carriage affected the growth of *E. coli* K12 strains, but not APEC. Interestingly, plasmid carriage played no role in biofilm formation in either genetic background. However, plasmid carriage influenced carbon, nitrogen, phosphorus and sulphur utilisation. Interestingly, the rate of plasmid transmission from one APEC to another increased with pH, although plasmid transmission was totally suppressed at pH 4. The association and invasion assays revealed that APEC with and without ESBL plasmids associated and invaded both Caco-2 and HD11 cells.

The studies described here indicate that ESBL plasmids can be mobilised to unrelated APEC strains. APEC harbouring ESBL plasmids were able to intimately associate and invade Caco-2 and HD11 cells. Furthermore, the phenotypic data generated in these studies suggests that ESBL plasmid carriage may influence the fitness of pathogenic and non-pathogenic *E. coli*.

HP03: Isolation of a Kunitz-type protein that interacts with *Fasciola cathepsins*-L

Lucianna Vaccaro[1], Maria J. Perteguer[1], Carolina Hurtado[1], Elizabeth Valdivieso[1], Laura Muiño[2], Victoria Martinez-Sernandez[2], Florencio M. Uberia[2], Teresa Gárate[1].

1 *Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220, Madrid, Spain.*
2 *Laboratorio de Parasitología, Facultad de Farmacia, Universidad de Santiago de Compostela, Spain.*

Cathepsins L are the main cysteine proteases produced by adult flukes. These proteases have been used as antigens in vaccination trials, considering that *Fasciola* adults basically depend on cathepsins L for feeding on host blood. The use in affinity-chromatography columns of MM3 monoclonal antibody, that specifically recognised *Fasciola* L-cathepsins, led us to the co-purification of a Kunitz-type *Fasciola* protein (FKTP) with the proteases. From the functional point of view, FKTP has been described to possess inhibitory activity against trypsin (Bozas *et al.*, 1995). It could be hypothesised that the presence of FHKT associated with *Fasciola* L-cathepsins may have an immunomodulatory function. As an alternative to a function in immune avoidance, a role of FHKT as inhibitor of cysteine proteinases can also be considered based on previous experiments in plants and ticks (Hansen *et al*., 2007; Sasaki *et al.,* 2006). In experiments in our laboratory we have demonstrated that rFKTP is also able of inhibiting human cathepsin-L activity. It should be interesting to ascertain the role of this cross-class inhibitor in the biology of *Fasciola* cathepsins.

This work was supported by the Spanish Ministry of Science and Innovation (AGL2006-13936-C02; AGL2010-22290-C03-0/02/03; AGL2011-30563-C03-03).

HP04: Identification of a novel Anisakis simplex serpin that inhibits human thrombin

Maria J. Perteguer[1], Elizabeth Valdivieso[1,2,] Pamela Colabúfalo[1], Carolina Hurtado[1], Esperanza Rodríguez[1], Paulino Gómez-Puertas[3], Florencio M. Ubeira[4], Teresa Gárate[1].

1 *Servicio de Parasitología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220, Madrid, Spain.*
2 *Laboratorio de Biología Celular de Parásitos, Instituto de Biología Experimental, Facultad de Ciencias, Universidad Central de Venezuela, 47069, Caracas 1041-A, Venezuela.*
3 *Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM) Campus UAM. Cantoblanco, 28049 Madrid. Spain.*
4 *Laboratorio de Parasitología, Facultad de Farmacia, Universidad de Santiago de Compostela, Spain.*

Several nematode protein inhibitors have been described as anticoagulant molecules. Most of them belong to the smapin family and have been fully characterised. Recently our research group identified a non-smapin nematode inhibitor with anticoagulant properties. The gene was isolated from an *Anisakis simplex* L3 cDNA library. The 43 kDa purified recombinant protein was called AniSerp and its biochemical and structural characteristics were investigated. AniSerp inhibits human thrombin in a dose-dependent manner and the effect is independent of heparin. Molecular dynamics studies suggest that AniSerp apparently inhibits thrombin via a suicide substrate-like mechanism, similar to the mechanism by which mammalian coagulation inhibitors act, such as heparin cofactor II and human antithrombin III. Although the AniSerp gene showed a signal peptide sequence the recombinant protein is not recognised by serum from patients suffering from anisakidosis.

This work was supported by the Spanish Ministry of Science and Innovation (SAF2002-04057-CO2-01) and the Instituto de Salud Carlos III within the project MPY 1404/09 and included in Innocash Program (Genoma España).

HP05: Novel hepatitis E-like virus found In Swedish moose

Jay Lin[1], Heléne Norder[2], Henrik Uhlhorn[3], Sandor Belak[1] and Frederik Widén[1]

1 *Department of Virology, Immunobiology and Parasitology (VIP), The National Veterinary Institute (SVA), Uppsala, Sweden*
2 *Department of Clinical Microbiology, Institution of Biomedicine, Sahlgrenska University Hospital, Gothenburg University, Sweden*
3 *Department of Pathology and Wildlife Diseases, The National Veterinary Institute (SVA), Uppsala, Sweden.*

The objective is to identify new hepatitis E virus (HEV) animal reservoirs that could contribute to viral zoonotic transmissions. Zoonotic HEV transmissions have been proven earlier from animals like swine, wild boar and deer. The moose (*Alces alces*) is hunted and consumed in Sweden and the possibility that moose carry HEV may reveal previously unexplored HEV transmission pathways for human infections.

One moose liver sample was HEV positive by real time PCR. Sequenced amplicon covering regions commonly used for genotyping revealed three open reading frames (ORF1-3) characteristic for HEV. The genomic sequence was highly divergent compared with existing HEVs. The moose HEV was phylogenetically deviated in its own branch, sharing the same ancestor with the human HEV-related viruses (genotypes 1-4) and the unclassified Japanese wild boar HEVs. Interestingly, the ORF3 exhibited the highest divergence. More profound genome sequence comparison to other HEVs will be presented.

The general HEV prevalence in many countries is unusually high ranging between 5-30 % and in Sweden it is estimated to 9 %. The HEV transmission pathways are often unknown. But it is suspected that animals are acting as viral reservoirs and spreading HEV by zoonotic transmissions. Many newly discovered animal HEV viruses have very divergent genomes, but their zoonotic properties have not been properly evaluated yet. *Our study confirms a* highly divergent *novel moose virus classified as a member in the Hepeviridae* family. Further investigations are necessary to clarify the moose HEV epidemiology, zoonotic potential and risk assessment for human infections.

HP06: Development of a challenge model for *Toxoplasma gondii* in cats: assessment of a dose-response for infection

Henk J. Wisselink[1], Jan B.W.J. Cornelissen[1], Peter F. M. Teunis[2], Katsuhisa Takumi[2], Joke W.B. van der Giessen[2]

1 *Central Veterinary Institute of Wageningen UR, P.O. Box 65, 8200 AB Lelystad, The Netherlands*
2 *Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), P.O. Box 1, 3720 BA Bilthoven, The Netherlands*

Toxoplasma gondii has a very wide intermediate host range and is capable to infect all warm-blooded animals. The cat is the definitive host and contaminates the environment through excretion of oocysts in faeces. A decreased excretion of oocysts will lead to a reduction of infections. For use in vaccination/challenge experiments in cats an experimental infection model was set up. To select a challenge strain we infected three groups of Swiss-Webster mice intraperitoneally with mouse tissue brain cysts (strains 76K or Gangji) or orally with oocysts (strain M4). The infection in the mice was followed by mortality, real time PCR, anti *T. gondii* antibodies, and the number of tissue cysts and bradyzoites in the brain. To infect cats, tissue cysts were isolated seven weeks after infection from the brains of M4-infected mice using a Percoll gradient, and bradyzoites were released by pepsin solution. Isolated bradyzoites (1000, 100, 50, 10) and tissue cysts (250) were used to orally infect five groups of three cats. Infected cats were monitored during three weeks for shedding of oocysts, as determined by classical faeces floatation techniques and real time PCR, and serology for anti *T. gondii* antibodies. After necropsy, tissue cysts production in heart, tongue and brains was determined. Real time PCR identified oocyst shedding in lower doses (up to 10 bradyzoites) compared to microscopical examination (up to 100 bradyzoites). In addition, shedding of oocysts, tissue cysts load in the tongue, heart and brains, and anti *T. gondii* antibodies was dependent of the bradyzoite dose. The results of this study can be used to perform challenge infections in cats.

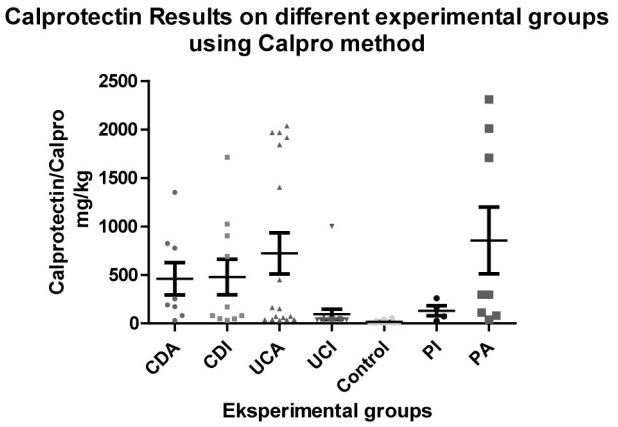
HP07: Relationship between serial changes in fecal calprotectin, clinical activity indices and C-reactive protein in patients with Inflammatory Bowel Disease

Mirsepasi H, Halkjær S, Holmetoft UB, Krogfelt KA, Petersen AM

Fecal calprotectin is a marker of inflammation in inflammatory bowel disease (IBD). Its usefulness in different IBD patient groups and as a marker of treatment efficiency is still debatable.

From 34 patients with ulcerative colitis, [16 active (UCA) and 18 inactive (UCI)], 18 patients with Crohn's disease, [8 active (CDA) and 10 inactive (CDI)], 12 with pouchitis, [8 active (PA) and 4 inactive (PI)], and 20 healthy controls (C) symptom scores, stool and blood samples were collected. Disease activity was measured with the colitis activity index (CAI), the Harvey-Bradshaw index (HBI) and the modified pouchitis disease activity index (MPDAI). In addition, fecal calprotectin, CAI and C-reactive protein (CRP) were measured in 4 serial stool samples from 11 UC patients with moderate disease activity during 12 weeks of treatment. Fecal calprotectin was analysed using the CALPRO calprotectin ELISA Test (ALP).

Fecal calprotectin concentrations are significantly associated with clinical disease activity indices in patients with ulcerative colitis, but not in patients with Crohn's disease or an ileo-anal pouch. Furthermore, fecal calprotectin was significantly lower in healthy controls compared to all patient groups, including ulcerative colitis patients in remission. In patients with active ulcerative colitis evaluated before and during 12 weeks of treatment, fecal calprotectin did correlate with the colitis activity index (CAI), both before, during and after treatment.



HP08: Investigating alternative fluoroquinolone-dosing approaches for preventing the selection of FQ-resistant mutants of *E. coli, Campylobacter* and *Salmonella* in poultry

Anne Ridley, Luke Randall, Becky Gosling, Rob Davies, Shaun Cawthraw, Liljana Petrovska, Charlotte Cook, Roberto la Ragione and Chris Teale

Animal Health and Veterinary Laboratories Agency, Woodham Lane, New Haw, Addlestone, Surrey. United Kingdom. KT15 3NB

Fluoroquinolones (FQ) remain important antimicrobials in human and veterinary medicine. However, current treatment regimens in poultry are associated with selection and persistence of resistance in enteric pathogens of public health importance. Together with prudent use, novel cost-effective approaches to FQ-dosing regimens, which prevent selection of resistant mutants, are required. A multidisciplinary study aimed at reducing the selection of FQ-resistant mutants of *Campylobacter*, *E. coli* and *S. Enterica* is described.

1.Mutation rate and MPC of 5 FQ, both alone and in combination with other poultry antibiotics, was determined *in vitro* for a comprehensive panel of target organisms. All FQs worked in an additive way with apramycin, colistin or lincomycin/spectinomycin. Apramycin reduced the enrofloxacin MPC by up to 8x that of enrofloxacin alone. Growth curves showed apramycin enhanced activity of enrofloxacin for sensitive organisms.

2. Sampling, in real time, of commercial turkey flocks medicated orally with enrofloxacin via drinking lines for disease treatment will investigate concentrations of the antibiotic in water, tissues and caecal contents of commercially reaered birds.

3. Data from above studies and from previously published data is being used to model pharmacokinetic and pharmacodynamic properties *in silico* to assist prediction of dosage regimens for preventing selection of FQ-resistant mutants.

4. Alternative treatment regimens will be investigated using tri-valent chick and turkey colonisation models. A preliminary study will investigate co-administration of standard enrofloxacin dose with apramycin in 22 day old chickens and turkeys colonised with *C. jejuni*, *S* Typhimurium and *E. coli*. Metagenomic approaches will detect mutations associated with FQR and changes in caecal flora during antibiotic treatment.

ES01: Large outbreak of *Salmonella* Thompson related to smoked salmon

IHM Friesema[1], AEI de Jong[2], DW Notermans[3], A Hofhuis[1], JHTC van den Kerkhof[4], R de Jonge[5], PM van Beek[4], MEOC Heck[3], W van Pelt[1]

1. *Epidemiology and Surveillance Unit, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven*
2. *Dutch Food and Consumer Product Safety Authority (NVWA), Utrecht*
3. *Laboratory for Infectious Diseases and Perinatal Screening, Centre for Infectious Disease Control, RIVM, Bilthoven*
4. *Preparedness and Response Unit, Centre for Infectious Disease Control, RIVM, Bilthoven*
5. *Laboratory for Zoonoses and Environmental Microbiology, Centre for Infectious Disease Control, RIVM, Bilthoven*

On 15 August 2012, the National Institute for Public Health (RIVM) noticed an increase in the number of *Salmonella* Thompson cases in the national surveillance, and an outbreak investigation was started. The regional public health services interrogated the cases with a semi-structured questionnaire about relevant exposures in the 7 days prior to the onset of symptoms. A similar questionnaire was sent to random control persons. Statistical analysis showed smoked fish from a specific coordinating supermarket organisation to be the problem (24 September). The Dutch Food and Consumer Product Safety Authority (NVWA) performed a trace-back of the incriminated product, inspected the suspected producer and took samples that showed contamination for *Salmonella* of smoked salmon, later to be confirmed *S*. Thompson, identical to that found in patients by molecular typing. Subsequently (28 September), all smoked salmon and derivatives of the producer in question were recalled. At the end of the outbreak, 31 December 2012, 1149 cases of *S*. Thompson were confirmed and four elderly persons were reported to have died. Results from an ongoing survey indicate that almost half of the Dutch people eat smoked salmon at least once per month. The incriminated producer had a large market share, thus several millions were potentially exposed. It is not clear how the prevention of large outbreaks involving unsuspected food products like *Salmonella* in smoked salmon can be improved. However, it stresses the necessity of high-quality laboratory surveillance of human cases both for "early" warning as well as effective outbreak investigation.

ES02: Multiple-locus variable number tandem repeat analysis of *Salmonella enterica* subsp. enterica serovar Dublin.

Marianne Kirstine Kjeldsen[1,2], Mia Torpdal[1], Karl Pedersen[2], and Eva Møller Nielsen[1]

1. *Foodborne pathogens and Bacterial Typing, Department of Microbial Surveillance and Research, Statens Serum Institut, Denmark*
2. *Zoonosis Center, National Food Institute, Technical University of Denmark, Denmark*

Salmonella Dublin is the most frequently isolated organism in clinical cases of cattle and infection causing diarrhoea, abortion, decreased milk yield and fatality. In humans, the serotype causes severe invasive disease, and high mortality rates have been reported. Epidemiological investigation of *Salmonella* benefits from molecular typing tools such as pulsed-field gel electrophoresis (PFGE), but this method provides limited diversity within *S.* Dublin. In this study we developed an MLVA protocol for *S. Dublin*. The polymorphism of nine potential VNTRs was evaluated with a panel of 40 diverse isolates. Four VNTRs were selected for an MLVA analysis and the discriminatory power of these was evaluated on 272 veterinary and human isolates and compared with that of PFGE. The four VNTRs exhibited 100% *in vitro* stability and contained only true repeats. When analysing 105 isolates MLVA obtained 58 genotypes and displayed a high level of discrimination (DI of 0.98) whereas PFGE grouped the isolates into 10 genotypes and displayed a low level of discriminatory power (DI of 0.57). MLVA divided the 272 isolates into 103 genotypes and successfully identified isolates from an epidemiological confirmed outbreak. The technique showed a significant enhanced discriminatory power when compared to the current 'gold standard' PFGE and can be recommended to be used in routine subtyping of isolates for outbreak investigations and disease surveillance. The method may provide valuable additional information that can improve the effectiveness of epidemiological investigations of *S.* Dublin infections and contribute to the efforts to reduce transmission of infection.

ES03: Development of a European molecular typing database for food, environmental and veterinary *Listeria monocytogenes* strains

Benjamin Félix[1], Damien Michelon[1], Pia Makela[2], Ivo Van Walle[3], Bertrand Lombard[4], Anne Brisabois[1], **Sophie Roussel[1]**

1. *University Paris-Est, Anses, Laboratory for Food Safety, European Union Reference Laboratory for Listeria monocytogenes, Unit Bacterial Characterization and Epidemiology, Maisons-Alfort, France*
2. *Unit of the Biological Monitoring Unit, EFSA, European Food Safety Authority, Via Carlo Magno 1A43126 Parma, Italy*
3. *ECDC, European Centre for Disease Prevention and Control, Food- and Waterborne Diseases and Zoonoses Programme, Tomtebodavägen 11 A, SE-17183 Stockholm, Sweden*
4. *University Paris-Est, Anses, Laboratory for Food Safety, European Union Reference Laboratory for Listeria monocytogenes, Maisons-Alfort, France*

The Anses Maisons-Alfort Laboratory for Food Safety has been designated European Union Reference Laboratory (EURL) for *Listeria monocytogenes* (*Lm*) by the Directorate-General for Health and Consumers of the European Commission. It coordinates a network of 35 National Reference Laboratories (NRLs). The EURL has set up a database for *Lm* (EURL *Lm* DB) which includes typing results as well as epidemiological information related to strains isolated from food samples. The EURL Lm DB is shared within the NRL network. Its objective is to gather data sets on *Lm* typing and related epidemiological data in the food chain. We describe here (1) the EURL typing activities which led to the setting up of the EURL *Lm* DB, (2) the use of this database, in view of improving public health. Currently the database is used to enter and share the typing data obtained in the frame of the European coordinated monitoring programme on the prevalence of *Lm* in certain ready-to-eat foods in the EU Member States and to analyse these typing data. In the frame of a close cooperation with ECDC, EFSA and SSI, these food typing data will be compared with those on human strains isolated during the same period of time, to allow a better estimation of the importance of certain foodstuffs as source of human listeriosis.

Used in combination with epidemiological investigations and databases on human strains, the EURL *Lm* DB should be a powerfull tool included in the surveillance system for improving detection of *Lm* strains circulating throughout Europe.

ES04: Economic assessment of surveillance in a One Health context: a research project on the impact of zoonotic disease surveillance

Sara Babo Martins[1,2], Jonathan Rushton[1], Katharina DC Stärk[1,2]

1. *Veterinary Clinical Sciences, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield AL9 7TA, UK.*
2. *SAFOSO AG, Bremgartenstrasse 109a, CH 3012 Bern, Switzerland*

One Health frameworks promoting inter-sectoral collaboration between the animal health and public health sectors have been increasingly recognised as needed to effectively address the threat of zoonotic diseases. For some zoonoses, it has already been shown that the control of disease in its animal reservoir is economically beneficial to the public health sector, but the same analysis has not been carried out addressing explicitly surveillance. While surveillance of zoonotic diseases in animals is anticipated to translate into economic benefits for the public health sector, it is also likely that different surveillance designs, the biology of the hazard under consideration and country settings will impact the results.

This 3-year research project (2013-2015) aims to inform public health and animal health decision makers on how to set priorities in resource allocation to surveillance of zoonotic diseases. A framework for the assessment of the economic impact from a public health perspective of different zoonosis surveillance designs in the animal population will be developed and applied to 3-5 case studies, using different diseases and different geographic and resource settings.

The results obtained from this work will provide both technical and economical advances in the conduct of surveillance of zoonotic diseases in animal hosts and enhance the information available on the economic benefits of One Health approaches to surveillance.

ES05: Modernisation of meat inspection for pigs

Lis Alban, Goncalo Pacheco, Amanda Brinch Kruse, Jesper Valentin Petersen

Danish Agriculture & Food Council and University of Copenhagen

The classical zoonotic infections bovine tuberculosis and trichinosis are under control in most parts of the world. However, nowadays important zoonoses caused by e.g. Salmonella and Yersinia cannot easily be dealt with at traditional inspection. It has long been suggested to omit unnecessary palpation and incisions to limit the cross-contamination with Salmonella. However, what would we risk if we stopped the routine palpations and incisions? To address this, three risk assessments were undertaken following international guidelines. We looked at the impact of omitting routine palpation of mandibular and mesenterial lymph nodes and lungs, and the routine opening of hearts.

Data consisted of a comparison study involving 3,000 plucks, own collection of slaughterhouse samples sent for laboratory investigation, slaughterhouse statistics, literature and expert opinion.

The risk assessments show that visual inspection of these lymph nodes is not associated with any increase in risk for consumers. Regarding the heart, some cases of endocarditis will be overlooked. This has no impact on food safety, because the agents involved are not foodborne. For the lungs, it was estimated that 1/5-1/3 of embolic pneumonia cases might be overlooked. This is primarily an aesthetic issue, because of the limited foodborne impact of the involved agents. Still, it is recommended to do what is possible to detect and remove cases indicative of pyaemia including embolic pneumonia. Therefore, in case of doubt plucks should be palpated – or sent to the rework area for extended examination. Continued discussions about meat inspection is needed to ensure maximum food safety for the resources spent.

ES06: Modernisation of meat inspection to prevent zoonotic risks in beef

Liza Rosenbaum Nielsen[1], Francisco Calvo-Artavía[1], Lis Alban[1,2]

1. *Faculty of Health and Medical Sciences, University of Copenhagen*
2. *Danish Agriculture & Food Council*

Modernisation of meat inspection is up for discussion to ensure that resources are allocated to optimise food safety. Visual inspection is suggested as a way of minimising cross-contamination at slaughter with zoonotic agents, i.e. *Salmonella* and *E. coli*.

In cattle, the masseter muscles are incised routinely to detect the presence of the zoonotic parasite *Cysticercus bovis*. The associated human infection (taeniosis) is unwanted although it does not lead to clinical disease. Would food safety be reduced, if traditional inspection was replaced by visual inspection with respect to *C. bovis* in low-prevalent populations? Are there other ways of inspection that can be just as effective as the traditional approach?

This was investigated in three studies. Data originated from the Danish Cattle Database, the slaughterhouse database, literature and expert opinion. First, a case-control study was undertaken to identify risk factors in Denmark. Subsequently, a study based on register data was conducted to estimate the true prevalence and identify more risk factors for C. bovis. Next, a scenario tree was built to illustrate the effect of various risk-based surveillance programmes. These showed that using gender as a risk factor – and limiting inspection to female cattle – was almost as effective as inspecting all cattle. Gender was interpreted as an indicator for production system, because females are more frequently let on pasture, where they can get exposed to parasite eggs and become infected with *C. bovis*. This approach would save resources that could be spent on other risk mitigating measures improving food safety.

ES07: Marked reduction in prevalence of *Salmonella* in the Danish cattle population under effective surveillance and control programmes

Liza Rosenbaum Nielsen

Faculty of Health and Medical Sciences, University of Copenhagen

One decade ago, Denmark had widespread *Salmonella* Dublin infection in cattle herds. Apart from causing disease and economic losses in cattle farms, this bacterium leads to rare but severe human cases with high case fatality. Therefore, since 2002 surveillance activities and a control programme have been in place involving several periods with targeted efforts to reduce the prevalence. The routinely collected data have been stored in the Danish Cattle Database and provided a unique source of data for further analysis on infection dynamics previously not understood about *Salmonella* Dublin.

The aim of this study was to investigate duration of infection in cattle herds and risk factors affecting the duration of infection. Survival analysis was performed on an extract of data from all active herds in the Danish Cattle Database. It was found that duration over all the years of surveillance was on average 2 years, but duration was affected by centrally organised control campaigns and several risk factors such as herd size, location and factors indicating level of hygiene or management. The prevalence of infected dairy herds was reduced from approximately 25% to the current 7.8% over 11 years and continues to decrease. This is only possible, because the surveillance and control programmes have been organised using One Health principles including strong leadership, careful planning and collaboration between all important institutions (i.e. the livestock industry, the meat industry, the Veterinary & Food Administration, and relevant research institutions). Therefore this provides inspiration for other effective disease control programmes of relevant zoonoses.

ES08: Q Fever in Belgium

Samira Boarbi, Marcella Mori, Patrick Michel, Raïssa Bakinahe, Martine Marin, Damien Desqueper, Sylvie Malbrecq and David Fretin

1. *Bacterial Zoonosis of Animals, Operational Directorate Bacterial Diseases, Veterinary and Agrochemical Research Center, VAR-CODA-CERVA, Brussels, Belgium*

Q fever is a zoonosis caused by the gram-negative obligate intracellular pathogen *Coxiella burnetii*. Since its discovery, and particularly following the recent outbreaks in The Netherlands, *C. burnetii* appeared as a clear public health concern. In December 2009, the Federal Agency for the Safety of the Food Chain (FASFC) decided to set up a surveillance of Q fever in the Belgian goat industry. The main objectives of this study were: 1) to determine the prevalence of Q fever, and 2) to characterise at the molecular level the circulating bacterial strains. Two types of analysis were possible on bulk tank milk (BTM), a real-time PCR (RT-PCR) to detect the presence of the bacterial DNA and an ELISA to measure the serological response. Following a screening from December 2009 until January 2013, we conclude that Q fever is present in the dairy goat industry and therefore its follow-up is needed. We also have determined the diversity of *C.burnetii* genotypes in the Belgian goat industry. The genotype of the main strain found in approximately half of the goat field samples matched exactly with predominant strain circulating in The Netherlands. This caprine strain and one bovine strain together with the reference Nine Mile strain were used for the development of infection models (Mori *et al., submitted*).

ES09: A longitudinal study of *Salmonella* infection in four farrow-to-finish pig herds in England

Elizabeth Marier[1], Laura Powell, Renee Sheehan, Rob H. Davies, Alex J.C. Cook.

Animal Health and Veterinary Laboratories Agency (AHVLA)
Woodham Lane
New Haw, Addlestone
Surrey, KT15 3NB

The aim was to further understand the transmission of *Salmonella* from the breeding herd to their progeny and identify the riskiest time-point for control.

Four indoor breeder-finisher pig farms known to be positive for *Salmonella* were identified. On each farm, three cohorts with a minimum of five breeding animals were selected on three consecutive weeks shortly after being served. Each group of sows and their offspring was sampled at a maximum of 13 time-points between pregnancy to weaning and up to finishing stage.

Pooled faecal samples (25g) from animals kept in groups and individual faecal samples from single animals were collected and cultured for *Salmonella*.

Overall 352/1592 (22.1%) of samples taken from the 127 sows selected and their offspring were positive for *Salmonella*. Serovar Kedougou was mainly identified in sows while Typhimurium and Derby were more prevalent in the offspring. The serovars isolated in sows were usually also isolated in the offspring.

During lactation, an increased risk of *Salmonella* in the offspring was observed when their sow was *Salmonella* positive RR4.9 [CI_{95}2.4-10.0].

Salmonella was mostly found post-weaning. Sows and offspring were shedding different serovars at different time-points. New serovars may be picked up by the animal when in contact with different environments through various movements during the cycle. Some serovars practically inexistent in piglets became dominant post weaning. Therefore, the increased risk of *Salmonella* observed in the offspring may suggest the possibility of *Salmonella* transmission between the sow and her piglets during lactation, but the environment may also contribute to the contamination of the weaners.

ES10: Monophasic *Salmonella* Typhimurium-like 4,12:i:- and 4,5,12:i:- in Danish animal production and meat

Karl Pedersen[1], Hector Arguello[2], Gitte Sørensen[1], Ana Carvajal Ureña[2], Pedro Rubio[2]

1. *Technical University of Denmark, National Food Institute, Søborg, Denmark*
2. *University of León, Faculty of Veterinary Science, Department of Animal Health, Infectious Diseases and Epidemiology Unit, León, Spain*

Monophasic *Salmonella* 4,12:i:- or 4,5,12:i:- have emerged in recent years in several countries, both as zoonotic agents in animal production and as the cause of human foodborne infections. Such *Salmonella* types have close resemblance to *S.* Typhimurium and investigations have confirmed that they are derived from *S.* Typhimurium. In this investigation, the objectives were to examine the occurrence and prevalence of *S.* 4,12:i:- and *S.* 4,5,12:i:- in Danish meat and food animal production samples from defined control programmes and herds. A subset of isolates was subjected to closer typing and characterisation by phage typing, pulsed-field gel electrophoresis, and MIC determination.

The results showed that monophasic *S.* Typhimurium-like *Salmonella* were common and constituted a significant proportion of the total *Salmonella* found, in particular in pigs and pork, where they constituted between 9.9 and 18.2 % of the total *Salmonella* isolates. In cattle they were found as the cause of clinical salmonellosis.

Investigation of 94 monophasic isolates showed that 60 were 4,5,12:i:- while 34 were 4,12:i:-. Among the 94 isolates, 68 were lacking the *fljB* gene, i.e. were genotypically monophasic, whereas 26 carried the *fljB* gene, but seemed not to express it.

The most common phage type was DT193 (n=61) followed by DT120. All isolates, irrespective of phage type had a marked tendency to being resistant to several antimicrobials, in particular to ampicillin, streptomycin, sulfonamides and tetracyclines, but not to cephalosporins or quinolones. Several clonal lineages were found by PFGE, but a single DT193 clone was dominant.

ES11: Slaughterhouse persistence of livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA ST398)

Marianne Sunde, Bjørn Lium, Jannice Schau Slettemeås, Madelaine Norström and **Anne Margrete Urdahl**

Norwegian Veterinary Institute, Oslo, Norway

During the last decade livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type (ST) 398 has emerged among pigs in European countries. Pigs are usually asymptomatic carriers, but MRSA ST398 can spread from animals to humans and therefore has a zoonotic potential.

Three studies on MRSA in swine in 2008, 2008 and 2012, have documented a very low prevalence of MRSA ST398 in Norwegian swine holdings with 0, 0 and 0.6% positive herds, respectively. However, in 2011, 1033 pigs from 207 different farms were sampled at ten different slaughterhouses. From one slaughterhouse, MRSA ST398 was detected in six pooled samples (3%). An attempt to identify positive swine holdings was performed unsuccessfully. Follow-up sampling of the environment in the slaughterhouse barn showed that MRSA ST398 was present in the environment, and thereby may have contaminated the pigs during housing at the slaughterhouse. For low prevalence countries like Norway, slaughterhouse sampling can give an overestimation of herd prevalence, and caution should therefore be taken using the results for further risk assessment on the importance of MRSA ST398 positive swine holdings.

The objective of the present study is to perform a follow-up sampling at the slaughterhouse, now two years after, to investigate whether the slaughterhouse environment still is contaminated with MRSA ST398. Results from this sampling will be presented and further discussed.

ES12: Source attribution of human *Salmonella* infections in Italy

Lapo Mughini Gras[1,5], Federica Barrucci[2], Joost H. Smid[3], Caterina Graziani[1], Ida Luzzi[4], Antonia Ricci[2], Lisa Barco[2], Roberto Rosmini[5], Arie H. Havelaar[3,6], Wilfrid van Pelt[3], Luca Busani[1]

1. *Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy.*
2. *Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy.*
3. *Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.*
4. *Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy.*
5. *Department of Veterinary Medical Sciences, Bologna University, Bologna, Italy.*
6. *Institute for Risk Assessment Sciences, Utrecht University, The Netherlands.*

We aimed at estimating the proportions of human *Salmonella* infections in Italy attributable to *Gallus gallus*, turkeys, pigs and ruminants, at the points of animal reservoir (farm), exposure (food), and both combined. A modified Dutch source attribution model, incorporating prevalence uncertainty and food consumption weights, was adapted to Italian *Salmonella* surveillance data of the top 30 serotypes in 2002–2010. Estimates were thus compared between time periods and sampling points.

Pigs were identified as the main source of human salmonellosis in Italy, accounting for 43% (95%CI: 42–44%) of infections at farm level, 45% (44–46%) at food level, and 44% (43–45%) at both these levels combined, followed by *Gallus gallus* (farm: 34%, 32–35%; food; 32%, 31–33%; farm+food: 33%, 32–34%), turkey (4%, 4–5% at all levels) and ruminants (2%, 2–3% at all levels). Human infections estimated to be travel- and outbreak-related amounted to 16% (15–17%) and 1% (1–1%), respectively. A significantly decreasing temporal trend in human infections attributable to *Gallus gallus*, and an increasing one of those attributable to pigs, was observed. This suggested that control measures have been successful in poultry but there is an urgent need to focus attention on pigs. Our attribution estimates are valid as a first indication of which sources are becoming increasingly important in Italy and are expected to be useful for the delineation of future risk management strategies, providing valuable insights about potential targets along the production chain.

ES13: Investigating animal reservoirs of diarrheagenic *E. coli* using multilocus sequence typing

Marie Anne Chattaway, Michaela Day, Claire Jenkins, Neil Woodford and John Wain

Public Health England, Colindale, UK (formally Health Protection Agency)

Diagnostic tests for the detection of Enteroaggregative *E. coli* (EAEC) and Enteropathogenic *E. coli* (EPEC) samples are not performed in routine diagnostic laboratories in the UK and epidemiological data on the source of infection are scarce. In the *E. coli* multilocus sequence type (MLST) public database, there are twenty-four predominant ST lineages containing *E. coli* from both animals and human, six of which include EAEC (ST complex 10, 155 and 38) and EPEC (ST complex 10, 20 and 29).

The aim of this study was to test 368 ESBL isolates from human (108) and animal (260) sources using PCR targeting EAEC (*agg*R gene) and EPEC (*eae* gene) to identify potential zoonotic lineages. Eleven isolates (<1%) were a*gg*R positive, all from human sources, eight (72%) of which were from ST 10, 155 or 38 complexes. Thirty-two (9%) were *eae* positive of which 26 (81%) were from an animal source. One third of EPEC isolates were from eight complexes including ST Complex 10 and 155.

In this study ESBL EPEC was found in both animals and humans whereas ESBL EAEC was only found in humans, although lineages associated with EAEC were also found in animals with the absence of *agg*R. This study uses population structure of *E. coli* to trace lineages of potential zoontic ESBL *E. coli* for surveillence and epidemiological investigation.

ES14: Diversity of *Mycobacterium bovis* strains in France from 1978 to 2011

Hauer Amandine[1,2], Cochard Thierry[1], De Cruz Krystel[2], Karoui Claudine[2], Henault Sylvie[2], Biet Franck[1], Boschiroli Maria Laura[2]

1. *National Institute for Agricultural Research (INRA), UMR1282, Infectiologie et Santé Publique (ISP-311), F-37380 Nouzilly, France.*
2. *French Agency for Food, Environmental and Occupational Health Safety (ANSES), Unité Zoonoses Bactériennes (UZB), Maisons Alfort, France*

M.bovis is a zoonotic bacterium responsible for bovine tuberculosis (bTB) belonging to the *M.tuberculosis* complex. A national control programme launched in the sixties allowed France to reach the European bTB-free status in 2000. Nevertheless, we observe a prevalence increase at regional levels in recent years.

For better understanding the epidemiological dynamics of bTB we analysed the genotypes (spoligotyping-MLVA) of 3224 French *M.bovis* strains isolated from 1978 to 2011 from a wide geographical distribution and differentiated in 540 different types:

In 1978-1990, the types' variety was relatively low (39.6%) due to a limited number of isolates; however, from 1991 to 2000, when bacteriology became necessary for bTB confirmation, genotypic diversity was maximal (78.5%). From 2001 to 2011, even though the isolate number was maximal, the diversity decreased (41.6%) as most strains belong to epidemiological hot-spots where bTB is endemic.

Three spoligotype families with a wide geographical distribution are predominant: SB0120, SB0134 and SB0121. Specific VNTR profiles in each family are observed with variation in geographical distribution specificity.
A new clonal group exclusive to Southern France was identified, the F4-family, including 25 different spoligotypes. This group is characterised by the presence of a truncated QUB26-locus repetition and the absence of the spacer 33 in the DR locus.

The decrease of genetic diversity is concomitant with the farming professionalisation and breeds' specialisation. Clonal expansion in bTB hot-spots corresponds to extensive breeding conditions. Thus, the French control programme needs a re-evaluation to stop bTB spreading in these regions where *M.bovis* strains may also present increased virulence traits.

ES15: Occurrence of *Salmonella* spp. and enteropathogenic *Yersinia* spp. in Swedish wild boars (Sus scrofa)

Axel Sannö[1,2,3], Magdalena Jacobson[2], Gete Hestvik[1] and Anna Aspan[1]

1 *National Veterinary Institute, 751 89 Uppsala, Sweden*
2 *Swedish Agricultural University, Department of Clinical Sciences, Box 7054, 750 07 Uppsala, Sweden.*
3 *Email: axel.sanno@sva.se*

The Swedish, as well as the European wild boar (*Sus scrofa*) populations, are expanding and increasing in numbers. Can this constitute a health risk for humans and/or domestic animals? It is estimated that 60,000 wild boars were shot and consumed in Sweden last year. Only few studies have been done on Swedish wild boars as carriers of human pathogens while European studies show that wild boars have the potential to harbour a wide range of pathogens that cause serious illness among humans. Among these *Salmonella* spp., *Yersinia* (Y.) *enterocolitica*, *Y. pseudotuberculosis* and *E. coli* O157:H7 are of particular importance. Samples of tonsils, ileocaecal lymph nodes and faeces from 88 wild boars were analysed using a combination of culture and PCR techniques to detect these four pathogens. 10%, 20% and 20% of the sampled animals carried *Salmonella* spp., *Y. enterocolitica* and *Y. pseudotuberculosis* respectively. No *E. coli* O157:H7 was detected. 36 (40.9%) individuals carried at least one of the pathogens, 8 of these 36 individuals carried two or three of the pathogens and the pathogens were found to a variable degree in all of the four types of samples. This indicates that wild boars may be a source of infection for humans handling and/or consuming wild boar meat. Wild boar may also serve as a host that spreads pathogens to domestic animals on pastures. However, further studies are needed to evaluate the risks of transmission to domestic animals and/or to humans.

Acknowledgement: Wildtech project (EU 7th Framework Program for Research and Technological Development, grant agreement no. 222633)

ES16: Human adapted *Klebsiella pneumoniae* ST11 and ST147 resistant to tigecycline from pet animals

Cristina M. Ovejero, Laura Hidalgo, Belén Gutiérrez, Laura Carrilero, Alfonso Santos-López, Daniel Thomas-Lopez, Andreas Hoefer, Gloria Santurde, Carmen Martin-Espada, Bruno Gonzalez-Zorn.

Animal Health Department and VISAVET, Universidad Complutense de Madrid, Spain.

Tigecycline is the first of a new tetracycline class, the glycylcyclines. Its use is restricted to humans in hospitals for the treatment of complicated infections caused by resistant microbes. To date, the emergence of human isolates resistant to tigecycline has been limited. In *Klebsiella pneumoniae* the sole tigecycline resistance mechanism is associated with over-expression of the RND-like efflux pump AcrAB-TolC. The aim of this study was to characterise these interesting isolates.

PFGE showed that the clinical isolates were not clonal and they were typed using MLST. The isolates were identified as ST11 and ST147, both highly successful human *K. pneumoniae* lineages. In both strains the resistance to tigecycline (MIC > 4 µg/ml) reverted in presence of PA-betaN, a non-specific efflux pump inhibitor, showing that an efflux pump was involved in the resistance of the isolates. Sequencing, cloning and expression analysis showed that none of the efflux pumps involved in tetracycline or tigecycline resistance in Enterobacteriaceae to date was responsible for the tigecycline phenotype.

This study describes for the first time the identification of two human internationally successful clones resistant to tigecycline isolated from animals, which reveals new ways of spreading for bacteria resistant to these last resort antibiotics. Our results suggest that resistance to these molecules in *Klebsiella* is mediated by a new efflux mechanism, posing new and intriguing questions about the origin of tigecycline resistance in these isolates.

ES17: Staphylococcus aureus in wild animals: healthy carriers and genetic diversity

M. Concepción Porrero[1], Gregorio Mentaberre[2], Sergio Sánchez[3], Pedro Fernández-Llario[4], Susana Gómez-Barrero[1], Nora Navarro-Gonzalez[2], Emmanuel Serrano[2,5], Encarna Casas-Díaz[2], Ignasi Marco[2], José-Francisco Fernández-Garayzabal[1,6], Ana Mateos[1,6], Dolors Vidal[3], Santiago Lavín[2], Lucas Domínguez[1,]

1 *Centro de Vigilancia Sanitaria Veterinaria (VISAVET). Universidad Complutense, 28040 Madrid, Spain.*
2 *Servei d'Ecopatologia de Fauna Salvatge (SEFaS)-Wildlife Diseases Research Group. Departament de Medicina i Cirurgia Animals. Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain.*
3 *Instituto de Investigación en Recursos Cinegéticos (IREC), CSIC-UCLM-JCCM, 13071 Ciudad Real, Spain. (Current address: National Centre of Microbiology. Institute of Health Carlos III, 28220 Majadahonda, Spain)*
4 *Red de Recursos Faunísticos, Universidad de Extremadura, 10072 Cáceres, Spain*
5 *Estadística i Investigació Operativa, Departament de Matemàtica, Universitat de Lleida, 25003 Lleida, Spain.*
6 *Departamento de Sanidad Animal. Facultad de Veterinaria. Universidad Complutense, 28040 Madrid, Spain.*

Staphylococcus aureus is considered a commensal bacterium in animals and humans, but *S. aureus* carriage in wild animals has not been analysed. Swabs from skin and nasal samples (nares) were taken from Eurasian griffon vulture (n=40 samples/ 40 animals), Iberian ibex (n=260 samples /157 animals), mouflon (n=4 samples/2 animals), red deer (n=544 samples/ 273 animals) and wild boar (n=1395 samples/716 animals) and cultured on Baird-Parker agar with rabbit plasma fibrinogen; compatible colonies (one per sample) were confirmed by PCR. The presence of *mec*A and *mec*C genes and *spa* typing were performed in all isolates. In total, 247 isolates from 226 animals were recovered, the most of them from nasal samples (77.23%). Two isolates were *mec*A positive (0.008%). Sixty five *spa* types (including 27 new descriptions) were detected, the most frequent ones being t3750 (n=53), t548 (n=27) and t528 (n=18).

Several *spa* types were mainly isolated from a particular animal species (98.1% and 94.4% of spa type t3750 and t528 isolates were from wild boars and Iberian ibex, respectively; the 66.7% of isolates *spa* type t548 were from wild boar and 33.3% from red deer). These data suggested that some spa types could be host adapted. Both *mec*A-carrying isolates (t127 and t011) were from wild boars. Although the protocol applied was not specific for the detection of MRSA, this result points out the interest of monitoring MRSA in wildlife. Moreover, the detected MRSA genotypes are frequently associated with MRSA from livestock and humans, which could indicate contacts between different reservoirs. Further studies regarding bacterial dissemination are to be evaluated, including water as route of spreading from human and domestic habitats to wildlife.

ES18: Prevalence of feline bartonellosis and multilocus sequence typing of *Bartonella henselae* isolates in urban stray cats living in Milan, Italy

Lauzi Stefania, Dilda Francesca, Pollera Claudia

Department of Veterinary Science and Public Health, University of Milan, Via Celoria 10, 20133 Milan, Italy

Cat scratch disease is a worldwide zoonosis caused predominately by Bartonella henselae and, to a lesser extent, by B. clarridgeiae. Cats are the natural reservoir and vectors for B. henselae and B. clarridgeiae infections in humans. Genetic heterogeneity of B. henselae strains has been reported and multiple sequence types (STs) have been identified by the use of multilocus sequence typing (MLST). Particular sequence types have been more frequently associated with zoonosis than others. The aim of this study was to evaluate the prevalence of B. henselae and B. clarridgeiae infection in stray cats from Milan, Italy, and to explore the genotypes of the B. henselae population for the evaluation of the potential risk of transmission to humans. Whole blood samples collected from 89 stray cats were cultured and analysed by PCR. Sequence types of the feline B. henselae isolates were delineated using MLST. Bartonella henselae was detected in four (4.5%) cats and B. clarridgeiae was detected in one (1.1%) cat by PCR on blood samples. Co-infection by B. henselae type I and type II was identified in one cat. Four B. henselae isolates were cultured and were characterised as ST1 (2/4), ST5 (1/4) and ST8 (1/4), which are more commonly regarded as human associated or zoonosis associated STs. Typical feline associated B. henselae STs were not observed. Despite the low prevalence of B. henselae infection in stray cats from Milan, further investigations are needed to assess the risk for human health.

ES19*: Unifying the epidemiological and population dynamics of the monophasic *Salmonella* infections using whole genome sequencing

Liljana Petrovska[1], Thomas Connor[2], Simon Harries[2], Rob Davies[1], Anthony Underwood[3], Katie Hopkins[3], John Wain[3,4], Martin Woodward[5], Gordon Dougan[6] and Rob Kingsley[6]

1 *Animal Health and Veterinary laboratories Agency, New Haw, Addlestone, Surrey, UK*
2 *Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, UK.*
3 *Public Health England, 61 Colindale Avenue, London UK*
4 *Norwich Medical School, University of East Anglia, Norwich, United Kingdom*
5 *The University of Reading, PO Box 226, Whiteknights, Reading, UK*

S. enterica serovar Typhimurium is defined by somatic antigens O 4,[5],12) and H i:1,2 that are determined by the long chain polysaccharide (LPS) and flagella antigens, respectively. However, since 1990 there has been increasing incidence in many locations worldwide of human and animal infection with Salmonella with antigenic formula 4,[5],12:i:- and 4,12,:i:- (monophasic *Salmonella*). In the European Union these isolates are generally of phage type DT120 or DT193 and appear to be replacing DT104 as the dominant phage type associated with multi-drug resistance (MDR). Using next-generation sequencing (NGS) technology we have used whole genome sequence variation to determine the phylogenetic relationship of 127 monophasic Typhimurium isolates from animals in the UK from 1995-2010 and from human clinical cases of disease from 2007-2010 and compare these with the genomic sequence of 29 commonly isolated human and animal biphasic S. Typhimurium (4,[5],12:i:1,2) isolates. These data indicate a clonal expansion of a clade of *S.* Typhimurium beginning in about the year 2000, that are phylogenetically distinct from the epidemic DT104 clade and monophasic isolates prior to the year 2000. Comparative genomics identified two novel genetic islands and a region encoding multiple antibiotic resistance genes. The monophasic phenotype was due to multiple deletion events that occurred during the epidemic.

This study provided a scientific basis for the phylogenetic classification of monophasic *Salmonella* as S. Typhimurium and not another separate emerging multidrug resistant serovar as well as an insight into the development of Salmonella epidemics that can inform future control programmes.

ES20: Public health relevant *Salmonella* serovars in reptiles

Magdalena Zając, Dariusz Wasyl, Andrzej Hoszowski, Krzysztof Szulowski

National Veterinary Research Institute, Al. Partyzantow 57, 24-100 Pulawy, Poland

Reptiles are known as asymptomatic carriers of *Salmonella* classified into different subspecies and serovars. The study to determine *Salmonella* prevalence in exotic reptiles ran between 2010 and 2012 found 86.2% positive out of 697 tested samples, originating from snakes (357), lizards (278), tortoises (60), crocodiles (2), and reptile exhibition environments. 922 isolates belonging to 209 *Salmonella* serovars were found. The aim of the current study was to characterise reptile isolates belonging to the top-15 serovars found in humans. A total of 136 isolates representing nine serovars were identified: *S.* Agona (n = 43), *S.* Kentucky (n = 24), *S.* Newport (n = 19), *S.* Infantis (n = 18), *S.* Enteritidis (n = 12), *S.* Hadar (n = 10), *S.* Typhimurium (n = 7), *S.* Derby (n = 2) and *S.* Saintpaul (n = 1). A variety of XbaI-PFGE profiles representing various clones were found in *S.* Agona, *S.* Kentucky, and *S.* Newport. Those reptile-associated clones showed that *Salmonella* infection spread both horizontally and vertically and might be transferred by animal trade and exhibitions. Additionally, some *S.* Kentucky isolates found in carnivore reptiles shared the same PFGE profiles with multi-drug and high-level ciprofloxacin resistant isolates from turkeys. The occurrence of serovars common in poultry might be a result of feeding with contaminated raw meat or even whole 1-day old poults. Reptiles should be considered as possible vector for *Salmonella* transmission to human hosts. Due to popularity as pets, public awareness and education are required to prevent potential *Salmonella* infections in reptile breeders, distributors and owners.

ES21: Prevalence of foodborne pathogen in raw milk collected in northwest Italy (Liguria)

Razzuoli Elisabetta, Gennari Massimo, Rubini Danja, Dellepiane Monica, Delfino Silvana, Migone Laura, Schiavetti Irene, Tiso Micaela, Vito Guendalina, **Ratto Alessandra**, Ferrari Angelo.

Food Control Laboratory, Istituto Zooprofilattico Sperimentale PLV, Genova – Savona (Italy)

The sale of raw milk by self-service has been authorised in Italy since 2004, however, only limited data on safety are available in the literature.
The aim of this study was to evaluate the prevalence of foodborne pathogen in raw milk collected in Liguria in 2008-2012.

Twenty automatic distributors were sampled once a year in 2008-2009, and twice a year in 2010-2012. Microbiological analyses were performed according to the International Organization for Standardization (ISO). Contamination by *E. Coli* O157, *Salmonella* spp., *L. monocytogenes*, thermotolerant *Campylobacter* and *Staphylococcus* coagulase positive (Scp), was investigated in all samples.

No sample was detected positive for *E. Coli* O157, *Salmonella* spp. and *L. monocytogenes*, while one sample was positive for thermotolerant *Campylobacter* (0.6%).
These results confirm the data obtained in other European studies related to prevalence of *Salmonella* (0-2.9%), *Campylobacter* (0-6%) and *E. Coli* O157 (0-5.7%) in raw milk; however the value obtained for *Listeria* (0% of prevalence) is lower compared to other research (2.2-10.2%). Concerning Scp, it was identified in 23.8% of the total samples, with a median concentration of 280.00 M (IQR 200.00 – 551.00); this value significantly varied ($P<0.05$) in relation to the season (higher in autumn than in spring). These results could be linked to the probable presence of sub-clinical mastitis (index of poor animal health) in dairy cows. In conclusion, our study suggests that the risk associated to the raw milk consumption seems to be lower in Liguria than in other European countries.

ES22: Prevalence of *Coxiella burnetii* and risk of adverse pregnancy outcomes among women exposed to livestock: evidence from the Danish National Birth Cohort

Stine Yde Nielsen, MD (1,2)
Kåre Mølbak, Director, MD, DMSc (3)
Anne-Marie Nybo Andersen, Professor, PhD (4)
Bjørn Kantsø, Scientist (5)
Karen Angeliki Krogfelt, Head of Unit, Professor, PhD (5)
Niels Henrik Hjøllund, Associate professor, Consultant, PhD (1,6)
Tine Brink Henriksen, Professor, Consultant, PhD (2)

1. *Department of Occupational Medicine, Regional Hospital West Jutland, Gl. Landevej 61, 7400 Herning, Denmark*
2. *Perinatal Epidemiology Research Unit, Aarhus University Hospital, Skejby, Brendstrupgaardsvej, 8200 Aarhus N, Denmark*
3. *Dept. of Infectious Epidemiology, Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S*
4. *Section of Social Medicine, Department of Public Health, University of Copenhagen, Oster Farimagsgade 5, DK-1014 Copenhagen, Denmark*
5. *Dept of microbiological surveillance and research Statens Serum Institut, Artillerivej 5, 2300 Copenhagen S, Denmark*
6. *Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus, Denmark*
Email:nhhjollund@gmail.com

Q fever in pregnancy is suspected to be a potential cause of fetal morbidity and mortality. We aimed to quantify risk of infection in pregnant women occupationally and environmentally exposed to *C. burnetii* and to examine if Q fever during pregnancy or seroconversion were associated with adverse pregnancy outcomes.

The Danish National Birth Cohort collected blood samples from 100,418 pregnant women (1996-2002). We sampled 195 women with occupational exposure to livestock (veterinarians and female farmers), 202 women with domestic exposure (dairy cattle and/or sheep) and 459 unexposed women. Outcome measures were spontaneous abortion, preterm birth, birth weight and small for gestational age (SGA).
Samples were screened for antibodies against *C. burnetii* in a commercial enzyme-linked immunosorbent assay (ELISA). Positive samples were confirmed with an immunofluorescence (IFA) test.

IFA (cutoff: >=1:128): the proportion of seropositive women was higher in the occupationally exposed population (47.2 % seropositive, RR: 9.8; 95%CI: 6.4-15.2) and in the domestically exposed population (32.2% seropositive, RR: 6.7; 95%CI: 4.3-10.6) compared to unexposed women (4.8% seropositive).
We found no increased risk of spontaneous abortion, preterm birth or low birth weight among IFA positive women when compared to IFA negative women.
Seroconversion during pregnancy was found in 10 women; they all delivered live babies at term, two were SGA.

We found a high prevalence of antibodies to *C. burnetii* among pregnant women with occupational or domestic exposure to cattle and/or sheep compared to unexposed pregnant women. Our findings suggest that contact with livestock is a risk factor for *C. burnetii* in Denmark, but we found no increased risk of adverse pregnancy outcome in women with verified exposure to *C. burnetii.*

ES23: Assessment of the socio-economic impact of *Taenia solium* cysticercosis in Angónia district, Mozambique

Chiara Trevisan[1], Nicolas Praet[2], Alberto Pondja[3], Yunuss Assane[3], Pascal Magnussen[1] and Maria Vang Johansen[1]

1 *Section for Parasitology and Aquatic Diseases Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark*
2 *Institute of Tropical Medicine, Antwerp, Belgium*
3 *Faculty of Veterinary Medicine, Eduardo Mondlane University, Maputo, Mozambique*

Taenia solium cysticercosis is an emerging zoonosis causing both public health and agricultural problems in many low-income countries.

The objective of the study was to assess the burden of the disease in the Angónia district of Mozambique.

Based on a prevalence of epilepsy of 15.6% the number of people with neurocysticercosis (NCC) associated epilepsy was estimated at 21,828 (95% CR, 17,998-26,168), representing 6.6% of the total population. Of these, more than half had never received treatment. The number of adult pigs diagnosed with cysticercosis was estimated at 7,129 (95% CR, 6,401-7,879), which corresponded to 35% of the total adult pig population. The total annual costs due to *T. solium* cysticercosis were estimated at around 1 million euro (1,058,445 (95% CR, 671,138-1,570,446)) (3.2 euro per person per year). Of these 15% were losses due to pig production and 85% to direct and indirect costs caused by human cysticercosis. The annual monetary burden per case of NCC associated epilepsy amounted to 41 euro (95% CR, 24.30-62.0). The estimated average number of DALYs lost was 7.7 (95% CR, 4.5-12.0) per thousand persons per year.

Angónia district should be considered a high endemic area for *T. solium* cysticercosis, causing serious public health and agricultural threats which affect the livelihood of subsistence farmers by reducing dramatically their economic and societal well-being.

A One Health approach is essential to control this parasitic disease and to increase the wealth of the poorest.

ES24: Risk factors for the occurrence of extended-spectrum cephalosporinase producing *E. coli* in pig herds.

Vibe Dalhoff Andersen[1], **Vibeke Frøkjær Jensen**[1], Håkan Vigre[1], Margit Andreasen[2], Yvonne Agersø[1].

1. *Division of Epidemiology and Genomic Biology, National Food Institute, Technical University Denmark.*
2. *Danish Agriculture and Food Council, Pig Research Centre, Denmark*

Extended-spectrum cephalosporinase (ESC) is a major emerging antimicrobial resistance problem worldwide. However, evidence documenting the effect of its potential risk factors is scarce.

This study investigated the association between the occurrence of ESC-producing *Escherichia coli* (ESC-E. coli) in Danish pig herds and prescriptions of 3th and 4th generation Cephalosporins (Ceph. III/IV) together with other potential risk factors.

National databases (CHR and VetStat) were used to select medium to large integrated pig herds, stratified by number of Ceph. III/IV prescriptions within a specific 1-year period. 20 herds without Ceph.III/IV prescriptions (lower 75th percentile) and 19 herds with frequent prescription (upper 90th-99th percentile) were included in the study. Nine pooled faecal samples per herd, three from each section (sows/piglets, weaners, slaughter pigs) were obtained. ESC- E. coli was identified using standard selective methods. Management data were obtained from a questionnaire.

ESC-E. coli was found in the sow section of 18/19 Ceph. III/IV exposed herds and of 4/20 non-exposed herds (RR= 5; 95% CI: 2 – 11). Logistic regression showed a strong significant association between prescription patterns of Ceph. III/IV (p=0.0007) and ESC-E.coli in the sow section. The final model also included Treatment Proportion (TP) (p=0.046) of other systemic antimicrobials in the sow section.

The results underscore the importance of restricting the use of Ceph. III/ IV to control the emergence of ESC. ESC-E.coli was isolated from the sow/piglets section > 6 months after cessation of Ceph. III/IV use; the effect of TP indicates that co-selection could contribute to the maintenance of ESC.

ES25: Prevalence of canine leishmaniasis in northwest Italy (Liguria)

Dellepiane Monica, Razzuoli Elisabetta, Tiso Micaela, Vito Guendalina, **Ratto Alessandra**, Mignone Walter, Arossa Claudio, Ferrari Angelo.

Laboratory of Diagnostic, Istituto Zooprofilattico Sperimentale PLV, Genova – Savona – Imperia (Italy).

The aim of this study, supported by a regional grant, was to evaluate the prevalence of canine leishmaniasis in northwest Italy (Liguria).

Samples of serum, collected from 2011 to 2012 (Study 1) and obtained from 21,100 dogs, were analysed and the prevalence was determined for each considered district (203). The results were compared to the data of a research performed between 2002 and 2003 (Study 2).

Leishmaniasis is a complex parasitic disease, characterised by heterogeneous clinical-epidemiological features and a wide geographic range; with regard to the ligurian area, this pathology in dogs shows an endemic distribution. Our results indicate that the prevalence of this canine disease is comparable between Studies 1 and 2. Nevertheless, some districts of Savona have registered a significant increase in prevalence ($P<0.05$) in Study 2 respect to Study 1 (e.g. Alassio: from 4.74% to 7.5%; Andora: from 4.94% to 9.2%; Savona: from 3.17 to 5.9%, Varazze: from 3.31 to 7.5%). Furthermore, sporadic cases have been recorded along the east area of Genova, thus confirming the eastward diffusion ongoing over the last 20 years.

The growth rate of the prevalence could be explained by the recent climate changes, which may have produced the optimal environmental conditions for the proliferation of phlebotomus.

Finally, it could be hypothesised that the prevalence of canine leishmaniasis could represent a risk marker for human health.

ES26: Tick-borne encephalitis virus - seropositive cattle in Belgium: a risk-based screening for (TBEV) antibodies in bovine sera

Sophie Roelandt[1], Vanessa Suin[2], Flavien Riocreux[1], Sophie Lamoral[2], Yves Van der Stede[1], Steven Van Gucht[2], Bernard Brochier[2], Stefan Roels[1]

1 *Unit Coordination Veterinary Diagnostics, Epidemiology and Risk Analysis, Unit Data Management and Analysis, Unit of Pathology & Prionology - OD Interactions and Surveillance – Veterinary and Agrochemical Research Centre (CODA-CERVA) - Brussels, Belgium*

2 *National Reference Centre for TBEV – Service Viral Diseases- Operational Directorate of Communicable and Infectious Diseases - Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium.*

The risk of TBEV getting introduced into Belgium remains high. At present there are suspicions of incursions and wildlife foci within Belgium. Domestic animals are excellent sentinels for TBEV surveillance and early warning systems for this emerging zoonotic disease of public health importance.
A risk-based sampling for serological screening in cattle (n=650) was performed by CODA-CERVA, to maximise detection of low prevalence endemic foci in a predefined "risk zone" (Eastern Belgian provinces).
Serum testing was performed by the TBEV Belgian National Reference Centre WIV-ISP, by golden standard seroneutralisation (SN) test, and will be confirmed in a mouse neutralisation model (ongoing).
Using a conservative 1/15 cut-off titer, 17 bovines = 2.615% (95%CI: 1.39 – 3.84%) were detected as TBEV seropositive. Additionally, 6 bovines showed borderline (=suspicious) final results (1/10 < RFFIT-titer < 1/15), equal to 0.92% (95%CI: 0.19 - 1.66%). This amounts to a total TBEV seroprevalence in Belgian cattle between 2.61% - 4.29%.
Most seropositive animals (n=21) are localised in Wallonia, three came from Flanders. Bovines with borderline results were often located close to confirmed seropositive animals. The geographical locations roughly coincided with known Belgian hot spots for Lyme disease, where we would expect westward TBEV incursion from abroad. The animals originate from 10 herds, are autochthonous (no imports), older female beef cattle (>2 years), representing a prevalence conforming to the literature.
Based on this risk-based cross-sectional serological survey, obtained through One Health cooperation, there are now strong suspicions that Belgium may contain TBEV endemic foci. Confirmation of SN results in the mouse model is expected.

ES27*: Client-server based molecular surveillance networks aid in the detection and combat of zoonotic diseases

Vranckx Katleen, Janssens Koen

Applied Maths NV, Keistraat 120, Sint-Martens-Latem, Belgium

Molecular surveillance networks are very useful to rapidly share essential data, especially in the case of multidisciplinary diseases such as zoonosis. In order to detect a potential outbreak as early as possible, it is necessary to compare molecular typing data from strains isolated from human patients and animals. This is not always convenient in practice as strains from humans and from animals are often investigated in different labs. A client-server model is a very efficient model for multidisciplinary molecular surveillance networks.

In this study we will discuss the different options for a client-server setup for molecular surveillance networks using Cryptosporidium as an example. Cryptosporidium is a parasite that causes long-lasting diarrheal illness and may be life-threatening to immuno-compromised patients. Different Cryptosporidium species and strains cause a varying severity of illness and the distinction between different species and strains can only be done by molecular typing. The 18s rRNA gene and a gene encoding for a 90kDa glycoprotein (gp60) are sequenced at the client site and the resulting sequences are submitted and compared to a central BioNumerics® Server database.

For many zoonotic diseases, the detection of the causative organisms is already performed in routine diagnostics labs, but very often exchange of data only occurs if one lab suspects an outbreak. A client-server model makes it very easy to submit the available data to a central server where outbreaks can be detected more quickly and reliably. Furthermore, the data accumulating in this central database can be used to elucidate epidemiology and transmission.

ES28: Vector-borne infections: risk-based and cost-effective surveillance systems

Egil Andreas Joor Fischer[1], René Boedker[2], Agnès Leblond[3], Petter Hopp[4], Yves van der Steede[5], Katharina Stärk[6], Anders Lindström[7],Jörn Gethmann[8], Ellen Kiel[9]

1. *Central Veterinary Institute, Part of Wageningen UR, P.O.Box 65 8200AB, Lelystad, The Netherlands. egil.fischer@wur.nl*
2. *DTU-National Veterinary Institute, Bulowvej 27, 1790 Kbh V., Copenhagen, Denmark*
3. *INRA-EpiA, VetagroSup, 1 Avenue Bourgelat, 69280, Marcy l'Etoile, France*
4. *Norwegian Veterinary Insititute, Ullevalsveien 68, 0106, Oslo, Norway*
5. *CODA-CERVA, Groeselenberg 99, B-1180, Bruxelles, Belgium*
6. *Veterinary Public Health Institute, Schwarzenburgstrasse 155, 3097, Liebefeld, Switzerland*
7. *SVA, Ullsvag 2B, 751 89, Uppsala, Sweden*
8. *Friedrich Löffler Institute, Seestrasse 55, 16868, Wusterhausen, Germany*
9. *Uni-Oldenburg, Ammerländer Heerstrasse 114-118, 26111, Oldenburg, Germany*

Recent outbreaks of vector-borne diseases (VBD) highlight the need for effective surveillance systems for (early) detection of VBDs. Active surveillance, i.e. searching for infected individuals, is expensive for emerging vector-borne diseases, because of required repeated sampling. Furthermore, the introduction of an emerging disease will occur focal and at low initial prevalence. Focusing active surveillance using risk factors of disease may increase the sensitivity and improve the cost-effectiveness of the system.
We propose a dynamic system for risk-based surveillance of VBD based on these three pillars: 1) estimating risk of introduction, 2) risk of spread (modeling R_0) and 3) analysing routinely collected production and health related data. Together these three constitute an effective tool for targeted surveillance on time windows and areas. Each of the three components provides independent indices for the risk of outbreaks, and together provide a more accurate risk score, taking into account the potential for occurrence (risk of transmission and of introduction) and the probability that an outbreak is going on (syndrome and passive surveillance). This approach may greatly focus the costly active surveillance increasing the output of the surveillance effort.
Here we will present the first preliminary results of our efforts to combine risk of introduction, R_0 modelling and signals from production and health related data. We will show the methodology to combine these three different sources of information.

ES29: Mussels as indicators for viral risk in raw water

Anna Charlotte Schultz[1], Mette Myrmel[2], Anders Morten Hay Sørensen[1], Jakob Ottoson[3], Jonas Toljander[4], Ronnie Eriksson[4], Magnus Simonsson[4].

1 *National Food Institute, Technical University of Denmark, Denmark.*
2 *Norwegian School of Veterinary Science, Norway.*
3 *National Veterinary Institute, Sweden.*
4 *National Food Agency, Sweden.*

Direct quantification of viral levels in raw water tends to vary greatly between samples, which hampers quantitative microbial risk assessment. Mussels bioaccumulate viruses if present in surrounding waters, reflecting the average of viruses present in the water over time. The aim was to create a baseline of viral levels in blue mussels (*Mytilus edulis*) located in Scandinavian waters representing raw water sources. Monthly samplings were conducted of mussels located in vicinity to the mouths of Göta River (Sweden) and Glomma River (Norway) which both serve as raw water sources for several municipalities. In addition, samples of outlet water from the main sewage treatment plants located along the rivers were analysed for noroviruses. Samples of mussels were enumerated for E. coli, noroviruses, sapoviruses, astroviruses, rotaviruses and adenoviruses by real-time RT-PCR, and noroviruses were typed by sequencing.

Virus levels in mussels showed a seasonal variation with the GI levels typically being 0.5 to 1 log higher compared to GII peaking in January with 10^5 and 10^4 genome copies/gram of digestive tissue for GI and GII, respectively. In contrast to the E. coli, clear correlations were seen of virus levels in mussels towards air and water temperatures and to the number of norovirus illnesses confirmed in the population. We believe that such data can serve as a baseline for future studies, a baseline that will enable comparison of 'timely' changes in viral levels in mussels with changes in interventions in sewage water treatment and occurrence of heavy rain falls with possible sewage overflow.

ES30*: Attributing foodborne salmonellosis in humans to animal reservoirs in the European Union using a multi-country stochastic model

Leonardo Víctor de Knegt; Sara Monteiro Pires, Tine Hald

Epidemiology and Risk Modeling Group
Division for Epidemiology and Microbial Genomics
National Food Institute, Technical University of Denmark

A Bayesian modelling approach comparing the occurrence of *Salmonella* serovars in animals and humans was used to attribute salmonellosis cases to broilers, turkeys, pigs, laying hens, travel and outbreaks in 24 European Union countries. *Salmonella* data from animals and humans, covering the period from 2007 to 2009, were provided by the European Centre for Disease Prevention and Control, or obtained from studies and reports published by the European Food Safety Authority. Availability of food sources for consumption was derived from trade and production data from the European Statistical Office. Results showed layers as the most important reservoir of human salmonellosis in Europe, with 42.4% (7,903,000 cases, 95% credibility interval (CI) 4,181,000 – 14,510,000) of cases, 95.9% of which caused by *S.* Enteritidis. The second most important source were pigs, with 31.1% (5,800,000 cases, 95% CI 2,973,000 – 11,100,000), 63.1% of which were caused by *S.* Typhimurium. Country-specific results show laying hens as the most important source of salmonellosis in 13 countries and pigs in eight. In Finland and Sweden, most cases were travel-related, highlighting differences in the epidemiology of *Salmonella*, surveillance focus and eating habits across the EU. The relative importance of the sources of *Salmonella* is a dynamic feature, changing over time, and since conducting this study, target control actions in the European Union have already reduced the parcel of cases attributable to layers/eggs.

RR01*: Combined analysis of source attribution and case-control data to investigate risk factors for human campylobacteriosis of chicken, ruminant, environmental, pet and exotic origin

Lapo Mughini Gras[1], Joost H. Smid[2], Jaap A. Wagenaar[3,4,5], Alfred de Boer[4], Arie H. Havelaar[2,6], Ingrid H.M. Friesema[2], Nigel P. French[7], Luca Busani[1], Wilfrid van Pelt[2]

1. *Department of Veterinary Public Health and Food Safety, Istituto Superiore di Sanità, Rome, Italy*
2. *Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands*
3. *Department of Infectious Diseases and Immunology, Utrecht University, The Netherlands*
4. *Central Veterinary Institute of Wageningen UR, Lelystad, The Netherlands*
5. *WHO Collaborating Centre for Reference and Research on Campylobacter/OIE Reference Laboratory for Campylobacteriosis, Utrecht/Lelystad, The Netherlands*
6. *Institute for Risk Assessment Sciences, Utrecht University, The Netherlands*
7. *Molecular Epidemiology and Public Health Laboratory, Massey University, Palmerston North, New Zealand*

Case-control studies typically trace back the origin of human *Campylobacter* infections to the exposure, which may not point to the original reservoir. *Campylobacter* infections can be attributed to reservoirs using multilocus sequence typing (MLST)-based source attribution modelling. We performed a combined case-control and MLST-based source attribution analysis to investigate risk factors for human campylobacteriosis of chicken, ruminant, environmental, pet and exotic origin. *Campylobacter jejuni/coli* strains from 737 human cases in a case-control study comprising 3119 frequency-matched controls were typed using MLST. The asymmetric island model for source attribution estimated the probability for the sequence types (STs) found in human cases to originate from each of the reservoirs. Cases were split according to their attributed reservoirs. Reservoir-specific risk factors were investigated using logistic regression analysis. Most cases (~87%) were attributed to chicken and cattle. Chicken consumption increased the risk for chicken-attributed infections, whereas consuming beef and pork was protective. Animal contact, barbecuing in non-urban areas, tripe consumption, and never/seldom chicken consumption were risk factors for ruminant-attributed infections. Game consumption and swimming in household swimming pools in springtime increased the risk for environment-attributed infections. Dog ownership increased the risk for environment- and pet-attributed infections. Person-to-person contacts around holiday periods were risk factors for domestic infections with exotic STs putatively introduced by returning travellers. In conclusion, individuals acquiring campylobacteriosis from different reservoirs have different associated risk factors. By enhancing risk factor identification/characterisation, public health messages are targeted more effectively. *Campylobacter* MLST-based source attribution modelling can enhance significantly the outcome of classical case-control studies.

RR02: Detection of *Coxiella burnetii* in goat semen by PCR

M. Heijne[1], M.V. Kroese[1], B.J. van Rotterdam[2], M. Koene[1] and H.I.J. Roest[1]

1 Central Veterinary Institute, part of Wageningen UR in Lelystad, The Netherlands
2 RIVM in Bilthoven, The Netherlands

Coxiella burnetii is the etiologic agent of Q fever. In the 2007-2010 Dutch Q fever outbreak dairy goats were the cause of human infection. Little is known about the transmission routes within goat herds. Although aerosol transmission is thought to be the major infection route, no information is available on the risk of sexual transmission. This might be an important route as one male goat mates about sixty female goats. To investigate the possibility of sexual transmission we tested semen of male goats on Q fever positive farms.

Semen samples of nine goats from three different farms were collected. The farms were bulk tank milk positive for *C. burnetii* DNA and all male goats were vaccinated against Q fever. Eight goats were tested three times with an interval around six weeks. One goat was only tested once. A *C. burnetii* specific multiplex real-time PCR was used to test the samples. PCR results were all negative.

Our results suggest that the risk of shedding of *C. burnetii* in semen of vaccinated goats is small. No information was available about the infection status of these goats. In female goats and cattle the infection status prior to vaccination influences excretion of *C. burnetii*. Vaccination of infected animals does not prevent shedding of *C. burnetii*. Since nothing is known about the risk of excretion of *C. burnetii* in semen of goats, we believe it is important to share our data. Additional research with vaccinated and non-vaccinated male goats is needed.

RR03: Source attribution of human salmonellosis in low-prevalence countries: a Northern European model

Sara M. Pires[1], Leonardo de Knegt[1], Thomas Rosendal[2], Jukka Ranta[3], Anne Margrete Urdahl[4], Berit Tafjord Heier[4], Elina Lahti[2], Helene Wahlström[2]

1 National Food Institute, Technical University of Denmark
2 National Veterinary Institute, Sweden
3 EVIRA, Finland
4 Norwegian Veterinary Institute

Recently a source-attribution (SA) model based on microbial-subtyping data from 2006 to 2009 that estimated the relative contribution of food-animals to human salmonellosis in the European Union (EU) was published. The model included 24 member states (MS) and 4 sources (turkeys, broilers, layers and pigs). Its strength was that it allowed for attribution estimation in countries with varied data availability, including countries where sufficient data was not available to do SA at the country level. Results showed that the contribution of sources varied substantially between regions and countries. In the EU in general, layers were the most important source of salmonellosis (43.8%), followed by pigs (26.9%), and 9.2% of cases were reported as travel-related. In Northern Europe, the ranking of sources followed the same order but with lower proportions, and the proportion of travel cases was substantially higher (34.5%). This may be explained by differences in the epidemiology of *Salmonella,* and in surveillance and control programmes in different MS. Some of the Nordic countries differ from most MSs, as the prevalence of *Salmonella* in animals is very low. We hypothesise that including countries with such different *Salmonella* statuses has an impact on the final country estimates. In order to evaluate this impact we will apply the SA model to data from only Denmark (DE), Finland (FI), Norway (NO) and Sweden (SE) and also separately to FI, NO and SE. We aim to assess if the attribution estimates of these models are different from the model including all 24 MS.

RR04: Risk assessment of Rift Valley fever in Western Europe.

Egil Fischer, Clazien de Vos, **Aline de Koeijer**

Central Veterinary Institute (CVI) of Wageningen UR, Lelystad, The Netherlands

Following the epidemic of blue tongue (BTV-8) in Europe, the awareness of emerging vector-borne infections increased, and risk assessments of these infections became an important request from policymakers. A risk assessment of infectious disease is a challenge in itself, since combing the probability of introduction with the impact of a potentially exponentially growing epidemic create a challenging problem. The complexities of vector-borne infections only serve to increase the challenge.

To resolve these issues, we implemented a recently developed framework to guide the user through the risk assessment of vector-borne infections. We applied this framework to Rift Valley fever (RVF), in a two-day workshop with experts in the field, to assess all the various relevant aspects of this infection. It was concluded that an epidemic of RVF may occur in Western Europe, and may affect a very large number of animals, while remaining a very limited epidemic amongst humans. The probability of such an epidemic depends on the specific region addressed, and the uncertainties regarding the probabilities and size of the epidemic are huge. These uncertainties mainly arise from unknowns on the local vectors.

We found that the use of a standardised approach to risk assessment of vector-borne infections is helpful in quick assessments, but also in combining the various aspects like probabilities, uncertainties, exponential growth and impact. Since the risk of zoonotic vector-borne infections appears to be on the increase, such methods can help evaluate the real situation and compare the risks.

RR05*: Framework for risk assessment of exotic vector-borne livestock diseases

Clazien de Vos, Johan Bremmer, Egil Fischer, **Aline de Koeijer**

Central Veterinary Institute (CVI) of Wageningen UR, Lelystad, The Netherlands

Since the epidemic of blue tongue (BTV-8) in Europe, the awareness of emerging vector-borne infections has increased, and risk assessment of these infections became an important request from policy-makers. Risk assessment is a common tool nowadays, but vector-borne infectious diseases bring some extra aspects, making such risk assessments more complicated.

To solve these difficulties, we developed a framework guiding the user through the risk assessment of vector-borne infections. It uses a stepwise approach, guided by a list of questions and a checklist, thus helping the risk assessor to consider, or include, all relevant steps into the assessment. Sections of the evaluation can be approached separately or combined, depending on the specific risk question to be addressed.

The framework can be applied at various levels, but is specifically useful in the first steps of a risk assessment. It can be used for a quick qualitative assessment of the risk, for example in a workshop of experts. It will speed up a semi-quantitative assessment by guiding and structuring the assessment. Finally, this tool will also be useful in the first stages of building a detailled quantitative assessment. We applied the tool to Rift Valley fever (RVF). This is an ideal example because it incorporates all the complications of vector-borne infections such as multiple host species, multiple vector species and also the risk for public health. This test was successful and convinced us that the tool is sufficiently complete. It is practical for use in quick assessments, and as a basis for detailed evaluations.

RR06: Signalling and risk assessment of emerging zoonoses in The Netherlands

Kitty Maassen, Joke van der Giessen, Arjen van de Giessen

The National Institute for Public Health and the Environment, Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands

In the last decade The Netherlands has encountered some outbreaks related to (emerging) zoonoses. After the avian influenza outbreak in 2003 causing conjunctivitis amongst cullers and the emergence of livestock-associated MRSA in 2004, the world's largest outbreak of Q fever among people took place. These events emphasised the need for a more systematic approach of sharing and assessing signals for (emerging) zoonotic infections. A zoonoses risk analysis structure was developed and implemented consisting of several steps covering signalling, response (including upscaling), outbreak management and decision-making at the governmental level. Important is that at each stage in all teams experts from both human and veterinary health are involved. In the 'signalling forum zoonoses' a risk assessment is performed on the zoonotic signals that are brought in by the participants. This signalling forum is the basis of the structure and there is a regular meeting every month, and if necessary ad hoc meetings can be organised in case of an urgent signal. Experts from the key veterinary institutes (Animal Health Service, Central Veterinary Institute, Faculty of Veterinary Medicine, Dutch Wildlife and Health Center), the Netherlands Food and Consumer Product Safety Authority and the National Institute for Public Health and the Environment assess the risk and determine whether a follow-up action is desired. In this example of a One Health strategy next to the actual assessment of signals, the signalling forum strengthens the relationship, collaboration and communication between the human and veterinary health partners in The Netherlands.

RR07: Making sense of zeros: impact on human health risk estimates

Sofia Duarte; Anders Stockmarr; Maarten Nauta

1. *National Food Institute, Technical University of Denmark – Epidemiology and Risk Modelling*
2. *DTU Informatics*

In quantitative microbiological risk assessment (QMRA), risk estimates depend on prevalence of contamination and the microbial concentrations in food products. The probability distribution describing variability in concentrations and the method used to fit it to microbial data may therefore influence the accuracy of risk estimation. A challenge underlying the fitting is that a low concentration goes often undetected in plate counting, producing an "artificial zero" that should be differentiated from a "true zero" (from an uncontaminated product), while fitting a distribution. An accurate separation between the two types of zeroes may be essential for the characterisation of prevalence and distribution of concentrations, and therefore for an accurate risk estimation.

First, we developed a method (implemented in R) that does not assume a limit of quantification (LOQ), differentiates "true" and "artificial" zeroes and estimates both the prevalence and the parameters of a lognormal distribution of microbial concentrations from a set of raw plate count data. Then we performed a simulation study to analyse the effect of using different distributions and different fitting methods on the risk estimates, using an existing QMRA model on *Campylobacter* in broiler meat. We used the developed fitting method and compared it with three alternative methods. The analysis was performed at two different concentration scenarios and ten levels of prevalence. The results show that a zero-inflated distribution, preferably fitted without assuming an LOQ, is usually the best choice for an accurate risk estimation at different concentration and prevalence levels.

RR08: Source attribution via microbial subtyping – A study towards a more practical approach

Hannah Sharp, Lars Valentin, Annemarie Kaesbohrer

Federal Institute for Risk Assessment (BfR), Max-Dohrn-Straße 8-10, 10599, Berlin, Germany

Source attribution methods attribute cases of foodborne disease to the food vehicle or other source responsible for illness. Identifying and quantifying the contribution of the different zoonotic sources to human infections is important for reducing the exposure of the consumer to zoonotic pathogens. One of these methods is the microbial subtyping approach, the principle of which is to compare the subtypes of isolates from different sources (e.g. animals, food) with the same subtypes isolated from humans. Following this approach we analysed the structure and mathematical characteristics of a Bayesian source attribution model described by Hald and colleagues (2004), and subsequently modified by David and colleagues (2012).

This analysis led us to the proposition of a modified data-based source attribution framework, which avoids any convergence problems of the Bayesian approach by solving the model equations deterministically. The results are in good accordance with the results of the Bayesian framework and the model is able to cope with many different structured data sets. We analysed the impact of each data component on the model outcome and give insight into the requirements regarding the quality of data necessary for microbiologic source attribution. Additionally, the modelling set up allows for incorporating uncertainty in data via Monte Carlo simulation.
The modelling work will be presented with two different data sets on *Salmonella* from two different time periods (2004-2007 and 2010). The identified and quantified contribution of each source is compared and discussed for the different years.

RR09: Risk-based microbiological criteria for Campylobacter on broiler meat

A. H. Havelaar[1,2], A.N. Swart[1]

1 *Centre for Zoonoses and Environmental Microbiology*
National Institute for Public Health and the Environment
P.O. Box 1, 3720 BA Bilthoven, the Netherlands

2 *Division Veterinary Public Health*
Institute for Risk Assessment Sciences
Faculty of Veterinary Medicine
Utrecht University
P.O. Box 80 175, 3508 TD Utrecht, the Netherlands

We evaluate a critical limit of 1,000 cfu *Campylobacter* per gram of breast skin in the MC, with all of 5 samples required to comply. Assuming that all *Campylobacter* in non-complying batches are fully inactivated (e.g. by heating), the model predicts a 67-72% reduction in consumer risk, while 32-37% of all batches would not meet the criterion. The proportion of batches not meeting the criterion varies between 4% and 57% for individual plants. If the MC is implemented as a process hygiene criterion (PHC), plants with (high levels of) non-conforming batches will need to improve processing hygiene.

The costs of implementing a PHC to the Dutch poultry industry are uncertain, with a best estimate of approximately 2 million € per year. The benefits to the Dutch economy are reduced costs-of-illness in the order of 9 million € per year and public health benefits in the order of 400 DALYs. The benefits are even bigger if the consumers of exported meat are considered, or if the PHC would also apply to imported broiler meat. This suggests a European approach is more efficient.

RR10: Mechanistic analysis of zoonotic infection risks: exercises in dose-response modelling

Thomas J. Hagenaars

Central Veterinary Institute, part of Wageningen UR
P.O. Box 65, 8200 AB Lelystad, The Netherlands

In part due to the Q fever crisis, people living in the vicinity of livestock production in The Netherlands have become increasingly worried about the possible effects of large-scale livestock production on their health.

For that reason, both national and local authorities would like to have an assessment framework for zoonotic risks around livestock farms as a tool to support their policy-making in agriculture. However, due to the many scientific unknowns it is not easy to develop a framework that is useful and at the same takes the relevant uncertainties into account. In fact, it has been stated in a recent report by the Health Council of The Netherlands that scientific uncertainties at present preclude making clear statements about the public health risks in the vicinity of farms.

As a building block for a future framework I here discuss in some mathematical detail the modelling of dose-response. Subsequently, I will use widely accepted dose-response models to make some scientific statements about the possible effect on public health of measures to reduce bio-aerosol emission from farms.

RR11: Source attribution of foodborne ESBL-*E. coli* in Germany

Lars Valentin, Hannah Sharp, Bernd, Appel, Annemarie Käsbohrer,

Federal Institute for Risk Assessment (BfR), Max-Dohrn-Straße 8-10, 10599, Berlin, Germany

Extended-spectrum beta-lactamases (ESBL) confer resistance over a wide spectrum of ß-lactam antibiotics, including penicillins, 2nd-, 3rd- and 4th-generation cephalosporins. Furthermore, such bacteria are often resistant to other antimicrobial classes by associated resistance mechanisms. This can be challenging if human infections have to be treated. Also problematic is the fact that these resistance genes are often located on mobile elements which can be spread intra- and inter-species.

These days, there is an intensive discussion about the origin of ESBL-producing *E. coli* in humans. We estimated the contribution of different animal sources (broiler, fattening pigs, dairy cattle, fattened cattle) to human cases, using data from several ongoing studies within the German RESET project **[1].** Our model, derived from the well-established *Salmonella* source attribution model based on microbial subtyping data **[2],** uses information on typing of *E. coli*, isolated by selective media from humans and different animal reservoirs, by their ESBL-genes, phylogenetic groups and prevalence rates.

First attempts using preliminary data sets indicated that many human cases cannot be explained by the animal sources considered. Furthermore, it showed that typing information considered to define the subtypes was not sufficiently discriminatory to differentiate the contribution of the individual sources. The results indicate that all sources considered may contribute to the exposure of humans.

We will present model results from an updated German dataset for ESBL-producing *E. coli* including antimicrobial resistance patterns.
This approach can help to assess the role of foodborne exposure.

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Med-Vet-Net gratefully acknowledge sponsorship from the following organizations:

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Belgium: Veterinary and Agrochemical Research Centre (VAR) www.coda-cerva.be
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Denmark: Statens Serum Institut (www.ssi.dk)
Governing Board representative - Eva Møller Nielsen (EMN@ssi.dk)

Denmark: Technical University of Denmark (DTU) www.vet.dtu.dk and www.food.dtu.dk
Governing Board representative - Jørgen Schlundt (jors@food.dtu.dk)

France: French Agency for food, environmental and occupational health and safety | Agence nationale de securite sanitaire de l'alimentation, de l'environnement et du travail (ANSES) www.anses.fr
Governing Board representative - André Jestin (Andre.JESTIN@anses.fr)
Treasurer of the Association – Arnaud Callegari (MVNCOORD@anses.fr)

Germany: Federal Institute for RiskAssessment | Bundesinstitut für Risikobewertung (BfR) www.bfr.bund.de/
Governing Board representative - Bernd Appel (Bernd.Appel@bfr.bund.de)

Hungary: Veterinary Medical Research Institute of the Hungarian Academy of Sciences (VMRI) www.vmri.hu
Governing Board representative - Béla Nagy (bnagy@linux.vmri.hu)

Italy: Central Public Health Institute in Italy | Istituto Superiore di Sanita (ISS)
Governing Board representative - Umberto Agrimi (umberto.agrimi@iss.it)

Netherlands: Central Veterinary Institute of Wageningen University and Research Centre (CVI) www.cvi.wur.nl
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Norway: Norwegian Veterinary Institute | Veterinærinstituttet (NVI) www.vetinst.no/
Governing Board representative - Merete Hofshagen (merete.hofshagen@vetinst.no)

Poland: **National Veterinary Research Institute | Państwowy Instytut Weterynaryjny - Państwowy Instytut Babdwczy (PIWET) www.piwet.pulawy.pl**
Governing Board representative - Krzysztof Smietanka (ksmiet@piwet.pulawy.pl)

Spain: **The Complutense University of Madrid | Complutense University Madrid (UCM) www.ucm.es**
Governing Board representative - Lucas Domìnguez Rodrìguez (lucasdo@visavet.ucm.es)

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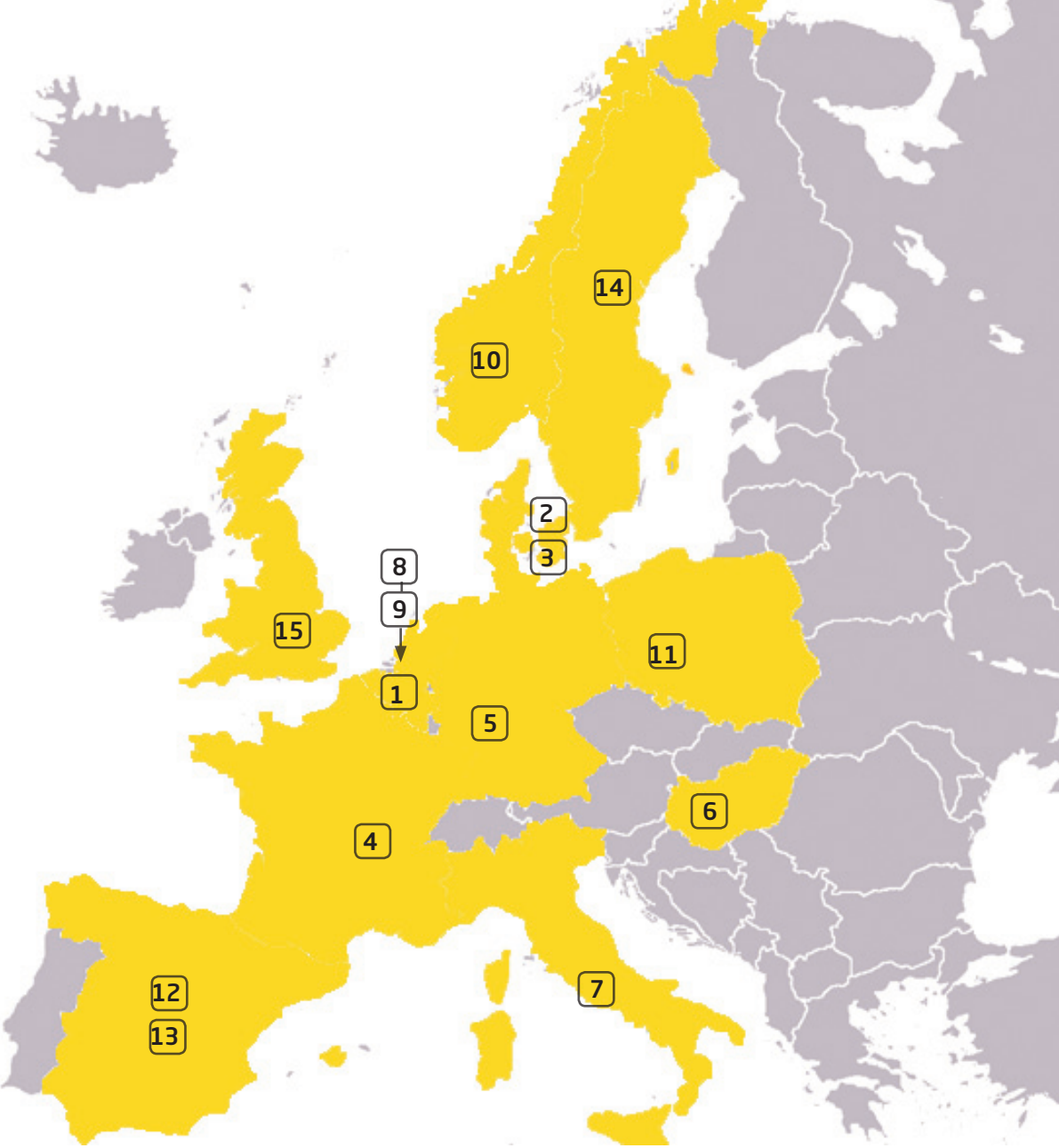
Governing Board representative – Roberto La Ragione (roberto.laragione@ahvla.gsi.gov.uk ; r.laragione@surrey.ac.uk)
Association Project manager – Anne Ridley (anne.ridley@ahvla.gsi.gov.uk)

Notes



1 Veterinary and Agrochemical Research Centre (VAR)

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